A State-of-the-Art Review of the Bioartificial Renal Assist Device Developed at the University of Michigan, USA

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Declaration

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

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Abstract

Over the last few decades, conventional haemodialysis has been the most successful choice of treatment for acute renal failure in intensive care units despite the mortality rate of 70 %. However haemodialysis still remains suboptimal as it does not replace all the lost functions of a normal kidney.

This thesis reviews an attempt involving cell therapy in combination with haemodialysis. In this method, a bioreactor containing kidney cells is incorporated in a conventional extracorporeal dialysis circuit with the aim of replicating metabolic and endocrine functions of the normal kidney. The thesis focuses on a revolutionary invention called the Renal tubule Assist Device (RAD) which is the result of research and study of a group of scientists led by Dr. H. David Humes in University of Michigan, USA. The thesis starts with an overview of the kidney's anatomy, physiology and fundamentals of the kidney functions. This is followed by explaining the design, mathematical modelling, *in vitro/ ex vivo* experiments and the first human trial. After reviewing the results of first human trial of the device, we discuss the outcomes, complications, criticisms and the areas of opportunities for further improvements. Finally, it comes to the point where the study stands at the moment, where is it leading in the future and how employing the current technologies and advancements could provide better treatment for patients with renal failure.

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CHAPTER ONE

INTRODUCTION

The current replacement therapies for treatment of Acute Renal Failure (ARF) or Acute Kidney Injuries (AKI), as it has been recently mentioned in the text books; do not compensate the loss of all kidney functions. Current methods such as dialysis or haemofiltration only substitute for the solute clearance function of the kidney and do not replace the lost reclamation, metabolic and endocrine function of this vital organ. The mortality rate of the patients with ARF is reported to be more than 50% in most studies (exceeding 70% in Intensive Care Units) (Feng and Humes, 2008). This has encouraged researchers to answer an important question - what is the exact role of live kidney cells that loss of them means decreasing the chance of survival in these patients?

The research group of Dr David Humes in the University of Michigan Medical School has developed a coupled system, comprising a conventional dialyser to carry out standard ultrafiltration and small solute removal and a novel cell-based technology to perform the metabolic process of the kidney. The latter bioartificial component is called the Renal tubule Assist Device or RAD. It has been demonstrated in large animal studies that the cells in the RAD perform metabolic and hormonal functions of the kidney that are lost in ARF (Sidorski and Peters, 1999). The phase I clinical trials of the RAD commenced in October 2004. A FDA-approved phase I/II trial was completed in April 2006 and showed significant clinical impact on survival and an acceptable safety profile of the RAD in treatment of AKI patients (Feng and Humes, 2008).

This thesis aims to review the design and performance of the RAD as well as explaining the tissue engineering and the future of this treatment.

1.1 Anatomy & Physiology of the Kidney

1.1.1 Anatomy of the kidney

Knowledge of the anatomy of the kidney is essential for a better understanding of the functions of this organ. In humans, the kidneys are situated on both sides of the vertebral column, behind the peritoneum, in the abdominal cavity. Each kidney is a

dark-red bean shaped organ about 12cm long and weighs about 150g. The top of each kidney is located at the level of the12th thoracic vertebra and the bottom at the 3rd lumbar vertebra. The left kidney is positioned slightly higher than the right one which is due to the presence of the liver pushing the right kidney down (Brenner and Rector, 1996).

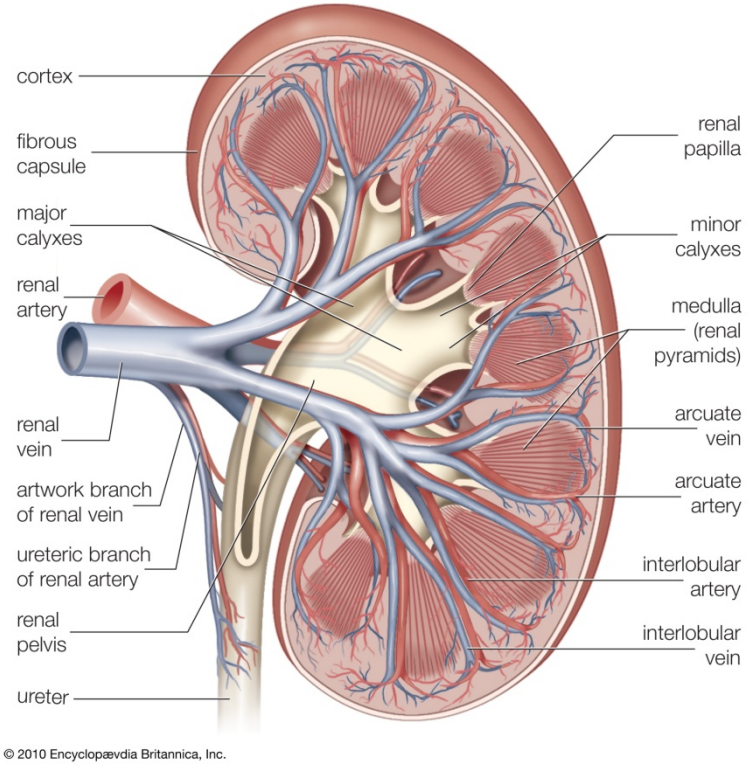

Figure 1.1 Kidney's cross section (Encyclopaedia Britannica Online, 2012a)

A cross-section of a human kidney is shown in Figure 1.1, on the medial surface of the kidney (concave side) the *hilus* is a convergence of the renal artery and vein, lymphatics, renal nerve and also the renal pelvis, which is the funnel-shaped end of the ureter. If a kidney was dissected from top to bottom, the cut surface would show two distinct regions, a dark outer region-the cortex- and a paler inner region-the medulla, which is further divided into a number of conical areas, the renal pyramids (Brenner and Rector, 1996).

Nephron

The basic functional unit of a kidney is the nephron (see Figure 1.2). Each human kidney has 1-1.5 million nephrons. The nephron is a blind-ended tube, the blind end forming a capsule (Bowman's capsule) around a skein of blood capillaries (the glomerulus). The other parts of the nephron are: proximal tubule, loop of Henle, distal tubule and collecting duct. As shown in Figure 1.2, the glomeruli, proximal tubules and distal tubules are located in the cortex, whereas the loops of Henle and the collecting ducts are extended down through the medulla (Brenner and Rector, 1996).

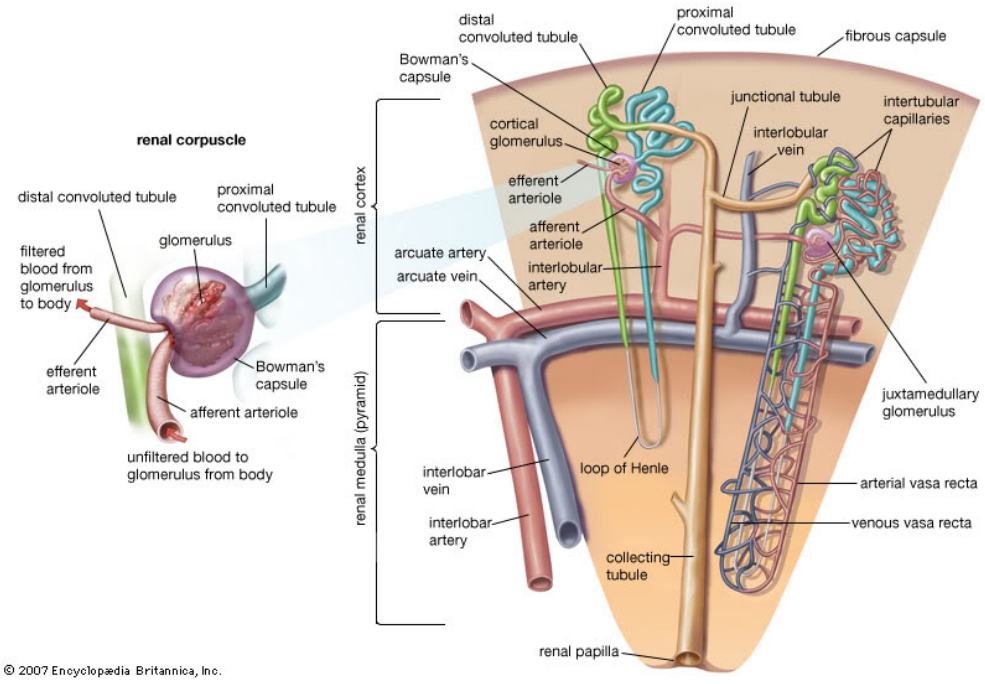

Figure 1.2 Different segments of a nephron within the cortex of kidney (Encyclopaedia Britannica Online, 2012b)

Glomerulus

The main role of the glomerulus is to ultrafilter plasma which is then introduced into the nephrons. The glomerulus has a diameter of about 200 μm. Each glomerulus is supplied by an ***afferent arteriole***, which divides inside the glomerulus to form the tuft of glomerular capillaries. Then these capillaries rejoin together to form the ***efferent arteriole*** (Brenner and Rector, 1996).

Proximal tubules

The morphology of the proximal tubule shows two distinct portions in its structure. The convoluted segment or *Pars Convoluta* which starts immediately behind the glomerulus and the straight segment or *Pars Recta*, which passes into the medulla to form the loop of Henle. The length of the proximal tubule in the human is generally about 15mm (12-25 mm range) with an outside diameter of 70 µm. As shown in Figure 1.3, the convoluted segment of the proximal tubule consist of columnar cells whose luminal surface has a "brush border" surface (microvilli) with a density of about 150 per µm² cell. This brush surface tremendously increases the surface area of the *Pars Convoluta* by a factor of about 40. The cells of the *Pars Recta* are very similar to those of convoluted segment, but have a less dense brush border (fewer microvilli), contained fewer mitochondria and are generally more flattened, suggesting that the transport functions of these cells are less developed than those of the *Pars Convoluta*. Hence the *Pars Convoluta* plays a greater role in absorption of tubular fluid compared to the rest of the proximal tubule (Brenner and Rector, 1996).

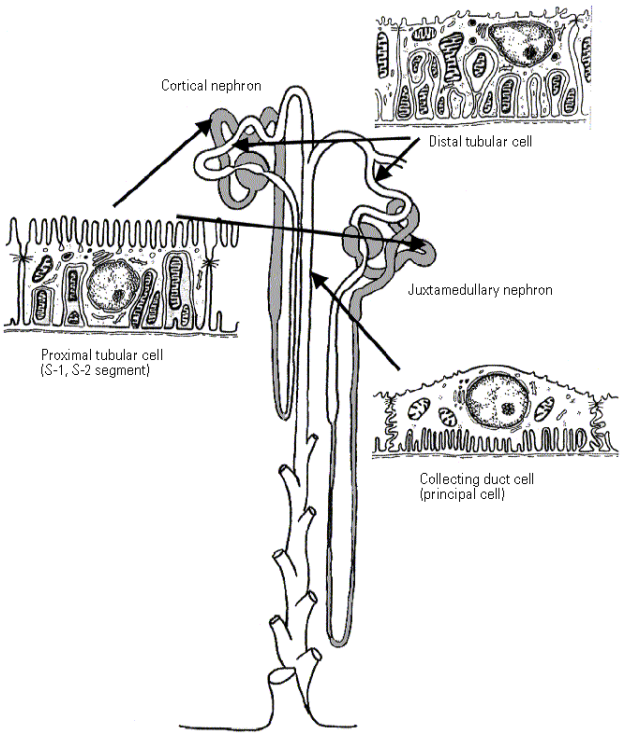

Figure 1.3 Schematic of two Nephrons (cortical and juxtamedullary) (Pfaller and Gstraunthaler, 1998)

1.1.2 Physiology of the Kidney

The kidney is a very complicated organ in terms of physiological processes and is responsible for:

- *Regulating the balance of water and inorganic ions* (e.g. Na, K, Ca...)
- *Removal of metabolic waste products*, so called because they are not beneficial to the body including; urea (from protein), uric acid (from nucleic acid break down), creatinine (from muscle creatine), the end products of haemoglobin breakdown, the metabolites of various hormones and many others
- *Excretion* which is the removal of foreign bodies like chemicals, drugs, pesticides, food additives...
- *Gluconeogenesis*: synthesizing glucose from amino acids to help liver in prolonged fasting periods (approximately 20% of the glucose being made by liver at the same time).

- *Secretion of hormones as an endocrine gland*
- *Immunoregulation* (Lote, 2000)

The basic renal functions are known as ***glomerular filtration***, ***tubular reabsorption*** and ***tubular secretion***. Urine starts to form as soon as the glomerular filtrate enters into Bowman's capsule. The bulk-flow of filtrate is called *glomerular filtration*. The glomerular filtrate contains no cells or plasma proteins and has almost the same concentration of most inorganic ions and low-weight organic solutes (like glucose and amino acids) as plasma.

The kidneys also act as ***endocrine glands*** secreting three important hormones:

- 1,25-dihydroxivitamin D_3 which plays an active role in calcium metabolism
- Erythropoietin which is involved in control of red blood cell production in bone marrow
- Renin which, as a component of the renin-angiotensin system, has a great effect on blood pressure control

Finally, and more related to this subject is the ***immunomodulatory*** role of the kidney in AKI. A growing number of studies have shown that inflammation plays a major role in the pathophysiology of AKI. In fact AKI is an inflammatory disorder to some extent (Feng and Humes, 2008). Studies have shown that the level of proinflammatory cytokines IL-6[1] and IL-8[2] in plasma predict mortality in patients with AKI (Simmons et al., 2004) (Wolff et al., 1998). On the other hand, the degree of inflammation in End Stage Renal Disease (ESRD) patients which is clearly a pro-inflammatory state, has been highly correlated to mortality rate (Bologa et al., 1998). These data suggest that renal function and most likely the renal tubule, play a critical immunomodulatory role in AKI (Feng and Humes, 2008).

[1] IL-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate immune response to trauma, especially burns or other tissue damage leading to inflammation

[2] Interleukin-8 (IL-8) is a chemokine produced by macrophages and other cell types such as epithelial cells. It is also synthesized by endothelial cells, which store IL-8 in their storage vesicles

1.2 Renal Failure and Complications

As mentioned earlier, the kidneys are responsible for maintaining the integrity of the internal environment, including volume, composition and distribution of the body fluids. The kidneys are primarily responsible for solute and water excretion with minor contributions from the lungs, skin and intestines.

Acute Renal Failure (ARF) is a syndrome that is characterized by a rapid decrease in Glomerular Filtration Rate (GFR) which can extend from hours to days. This syndrome causes retention of nitrogenous waste products, makes changes in extracellular fluid volume/electrolytes and acid-base homeostasis. The causes of ARF can be divided into three categories for the purposes of diagnosis and management (Longo et al., 2008):

(1) Diseases or events that cause renal hypoperfusion without compromising the integrity of renal parenchyma (prerenal ARF, prerenal azotemia) (~55%)

(2) Diseases that directly involve renal parenchyma (intrinsic renal ARF, renal azotemia) (~40%)

(3) Diseases associated with urinary tract obstruction (postrenal ARF, postrenal azotemia) (~5%).

Prerenal ARF is the most common form of ARF, although it is rapidly reversible if renal blood flow and consequently glomerular ultrafiltration pressure is restored. Cases with severe low renal blood flow may lead to ischemic injury of renal parenchyma and ultimately intrinsic renal ARF that altogether with prerenal causes are responsible for 95% of all ARF cases. Most ARF cases are reversible as the kidney is relatively unique among major organs in terms of recovery from almost complete loss of function. Nevertheless, ARF is associated with major in-hospital

morbidity and mortality, in large part due to the serious nature of the illnesses that lead to ARF (Longo et al., 2008).

1.2.1 Complications

AKI causes an imbalance in blood levels of many important elements such as sodium, potassium, acid-base mechanism and distribution of fluids through the whole body. As a result of this impairment, AKI usually is manifested by *intravascular volume overload, hyperkalaemia, hyperphosphataemia, hypocalcaemia, hypermagnesaemia, anaemia and metabolic acidosis.* In addition, as these patients are not able to excrete nitrogenous waste products, they are prone to develop uraemic syndrome (Longo et al., 2008). The speed of developing the impairment and the severity of such complications, defines the degree of the kidney failure and catabolic state of the patient.

Intravascular volume overload is an inevitable result of decrease in salt and water excretion in patients with reduced or no urinal function left. This can be noticed in milder forms as weight gain or oedema; in severe forms as life threatening cases of pulmonary oedema.

Hyperkalaemia is a typical sign of AKI which is caused by accumulation of potassium in blood due to lack of excretion from the kidneys and the release from damaged tissues as well as ingestion from food. Hyperkalaemia in mild form is almost symptom free but at higher levels can cause cardiac arrhythmia or even arrest.

Hypocalcaemia is often asymptomatic but can cause perioral paresthesia, muscle cramps, seizures, hallucination/confusion and changes in ECG.

Hyperphosphataemia or high blood level of phosphate in blood is almost inevitable in renal patients. Severe hyperphosphataemia causes deposition of calcium phosphate which can lead to hypocalcaemia, particularly when it is associated with other factors

like tissue resistance to the actions of parathyroid hormones which causes hyperthyroidism or low levels of active vitamin D (1,25-dihydroxyvitamin D).

Another common and important complication of AKI is anaemia which is usually mild and multi-factorial in nature. These factors include haemolysis (mechanical or chemical break down of red blood cells), bleeding, impaired erythropoiesis (the formation of red blood cells), haemodilution (a decrease in the proportion of red blood cells relative to the plasma), and reduced red cell lifespan.

Metabolic acidosis is initiated by accumulation of ions produced by metabolism of dietary protein, which normally is excreted by healthy kidneys. Metabolic acidosis can become more severe and complicated when production of endogenous hydrogen ions is increased by other mechanisms (e.g. diabetes, liver disease or sepsis). Extreme acidosis can lead to neurological (lethargy, stupor, coma or seizure) or cardiac (arrhythmia and low blood pressure) complications (Longo et al., 2008).

1.2.2 What is the cost of kidney disease?

De Vecchi et al (1999) compared the cost, as a percentage of total healthcare expenditure, of dialysis in six European countries, ranging from 0.7% in the United Kingdom to 1.8% in Belgium.

The National Service Framework (NSF) for renal services reported that in the UK the cost of treating End Stage Renal Failure (ESRF) patients is 1-2% of the national budget and this only comprises 0.05% of the population (Department of Health, 2004). The “NHS reference cost 2009-2010” (Department of Health UK, 2011) reported the cost of a single session of haemodialysis/filtration for a 19 years old and over patient (Day/Night) ranged from £138 to £188 and for a similar continuous ambulatory peritoneal dialysis (CAPD) patient the cost ranged from £38 to £52. The cost of a kidney transplant in adults varied from £14522 to £21532 for deceased donor (from heart beating to non-heart beating donor) and £17862 for a live donor (all these costs are when the kidney transplant is from a donor of 19 years old and

over) (NHS Kidney Care, 2011). In 2002 that the current spending on kidney care was about £445 million per year and would become even more with the advances in haemodialysis and improvements in primary and palliative care (Wanless, 2002).

1.3 Renal Replacement Therapy Methods

According to "The Renal Association"- UK- guidelines, the management of AKI treatment is based on:

- Assessment
- Prevention
- Pharmacological treatment
- Choice of Renal Replacement Therapy modality (RRT)

According to this guideline:

> "*Patients at risk of AKI should be identified in the community and the hospital. Risk factors should be identified and preventative measures instituted as early as possible. Prescription of appropriate intravenous fluids should be carefully considered following assessment of the patient's volume status"* (Davenport et al., 2008).

It is important to know that most of the risk factors for patients developing AKI include pre-existing chronic kidney disease (CKD), older age (>60 years old), sepsis, cardiac failure, liver disease, diabetes mellitus, and nephrotoxic medications. These risk factors can be detected during a prompt assessment and treated accordingly to reduce any risk of developing AKI.

In the light of knowing these risk factors, the majority of the cases can be effectively treated and resolved by treatment of the underlying disease. There is currently no evidence to support the pharmacological treatment of AKI; however some of the

treatments that are more common and believed to be beneficial are briefly reviewed here:

1.3.1 Pharmacological Treatment of AKI

Pharmacological interventions in AKI are focused on preventing the kidney falling into an ischaemic stage or developing inflammatory and hormonal problems. *Low-dose Dopamine* has traditionally been used and believed to improve renal perfusion and therefore prevent AKI, but recently a meta-analysis study (Friedrich et al., 2005) has shown that this treatment has no effect on mortality and RRT requirement. Similarly, Atrial Natriuretic Peptide (ANP), a hormone that increases glomerular filtration by dilating afferent and constricting efferent arterioles seemed to be a promising alternative for a pharmacological treatment. Anaritide, an ANP agent, has no effect on dialysis-free survival and causes increased rate of hypotension at the initial dose. However, in a small randomized study of 59 post-cardiac surgery patients with AKI, it was shown that lower doses can significantly increase the rate of 21-day dialysis free survival (Fieghen et al., 2009).

There are a number of other agents that have shown promising results in prevention or even early treatment of AKI. However the efficacy of these therapies is very dependent on the early diagnosis of AKI.

1.3.2 Renal Replacement Therapy

The choice of RRT modality is entirely related to every patient's medical status, medical/nursing expertise and also the availability of equipment. In the early 1980's, the choices for RRT therapy were limited to Intermittent Haemodialysis (IHD) and Peritoneal Dialysis (PD). Advances in biomedical technology and research made other therapies, such as Continuous Renal Replacement (CRR) and recently "Hybrid techniques", available to employ in the treatment of AKI patients. Renal replacement

therapy treatment can be used in different regimes based on the duration of treatment (Figure 1.4). For instance, the duration of a continuous therapy usually exceeds 24 hours while in an intermittent treatment it is less than 24 hours.

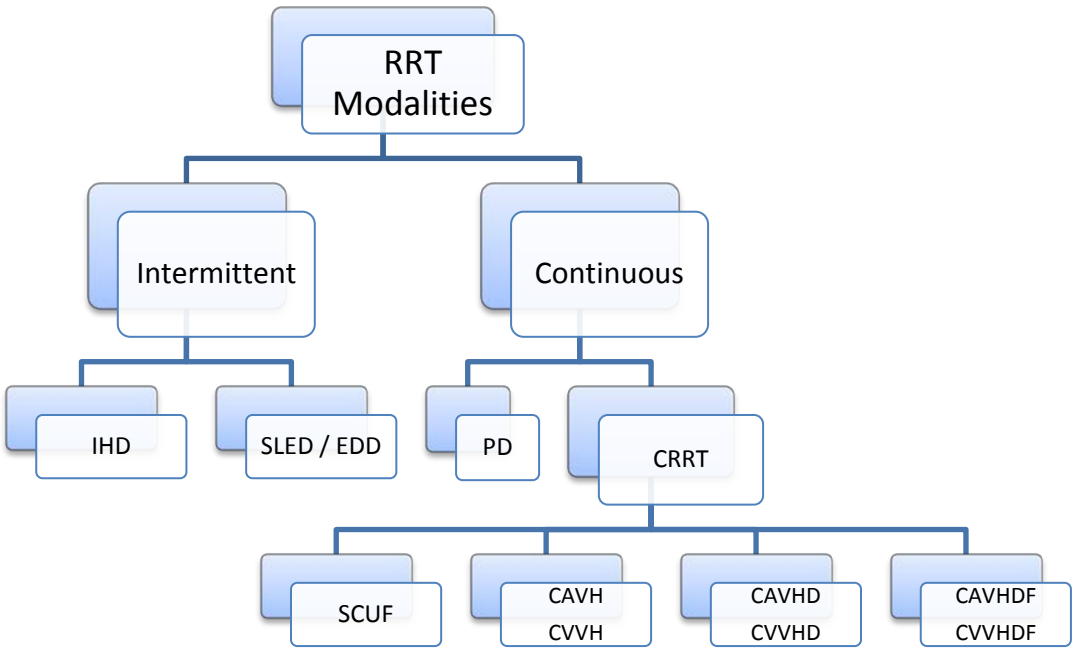

Figure 1.4 Renal replacement modalities for acute renal failure: CRRT, continuous renal replacement therapy; CAVH, continuous arteriovenous hemofiltration; CAVHD, continuous arteriovenous hemodialysis; CAVHDF, continuous arteriovenous hemodiafiltration; CVVH, continuous venovenous hemofiltration; CVVHD, continuous venovenous hemodialysis; CVVHDF, continuous venovenous hemodiafiltration; EDD, extended daily dialysis; IHD, intermittent hemodialysis; PD, peritoneal dialysis; RRT, renal replacement therapy; SCUF, slow continuous ultrafiltration; SLED, sustained low efficiency dialysis (O'Reilly and Tolwani, 2005).

1.3.3 Peritoneal Dialysis

In peritoneal dialysis, a volume of 1-3 litres of a Dextrose-based solution is inserted into the abdominal cavity and is allowed to remain for 2-4 h. The basics of toxin removal and fluid exchange, similar to haemodialysis, are through convective clearance by ultrafiltration and diffusive clearance via concentration gradient across a semi-permeable membrane, in this case the peritoneum.

The clearance of solute and water during peritoneal dialysis depends on the exchange rate across the peritoneum and this rate slows down with time and eventually stops

when equilibrium between plasma and dialysate solution is reached. This exchange rate also varies from patient to patient and can be affected by infection (peritonitis), drugs (beta blockers, calcium channel blockers...) or physical factors like position or exercise (Longo et al., 2008).

Peritoneal dialysis can be employed in different methods, such as:

- Continuous Ambulatory Peritoneal Dialysis (CAPD) is performed manually, and the solution is infused into the abdominal cavity 3-4 times a day.
- Nocturnal Intermittent Peritoneal Dialysis (NIPD): the same method as CAPD is applied except for having the solution infused at bed time and drained in the morning (in both methods, the infusion and drainage is based on gravity assistance).
- Continuous Cyclic Peritoneal Dialysis (CCPD): in this method, 4 or 5 fluid exchanges are performed with the help of an automated machine while the patient is asleep overnight.

The infusion of solution is via a temporary or permanent curved tube (Tenckhoff catheter) inserted into the abdominal cavity with side holes for equi-distribution of the solution. ESRD patients (or their carer) who decide to select PD as their preferred choice of dialysis are trained to perform the exchange at home with support from their PD clinic.

Peritoneal Dialysis in the form of Continuous Ambulatory Peritoneal Dialysis (CAPD) was introduced in late the 1970's. Despite the simplicity of PD and its ease of use at home, the popularity of PD has declined due to the fact that its efficiency decreases over time (Pierratos, 2004). Peritoneal dialysis is rarely used in critically ill patients due to insufficient solute clearance, risk of peritonitis, compromising the respiratory efficiency by leaving less space for diaphragm muscle expansion. It is contraindicated in situations of recent abdominal surgery or sepsis (O'Reilly and Tolwani, 2005).

1.3.4 Haemodialysis

Haemodialysis is typically referred to a method of treatment using a machine to treat acute and chronic renal failure patients. Haemodialysis is based on the principle of diffusion across a semi-permeable membrane. The metabolic waste products, based on concentration gradient, move from blood into the dialysate[3].

Based on the laws of diffusion, the larger the molecule is, the slower the rate of transfer across the membrane. Therefore, a small molecule like urea (60 Da) is cleared more quickly than a larger molecule like creatinine (113 Da).

The rate of solute transfer is influenced by the solute concentration in dialysate, the membrane surface area (*A*) and the overall mass transfer coefficient $k_{\circ}$. $k_{\circ}$ is a function of porosity and thickness of the membrane, the size of the solute molecules and the thickness of the blood and dialysate concentration boundary layers adjacent to the membrane.

[3] Dialysate: the fluid and solute in a dialysis process that passes through dialyser and is separated from blood by a semi-permeable membrane and is discarded along with the removed waste products

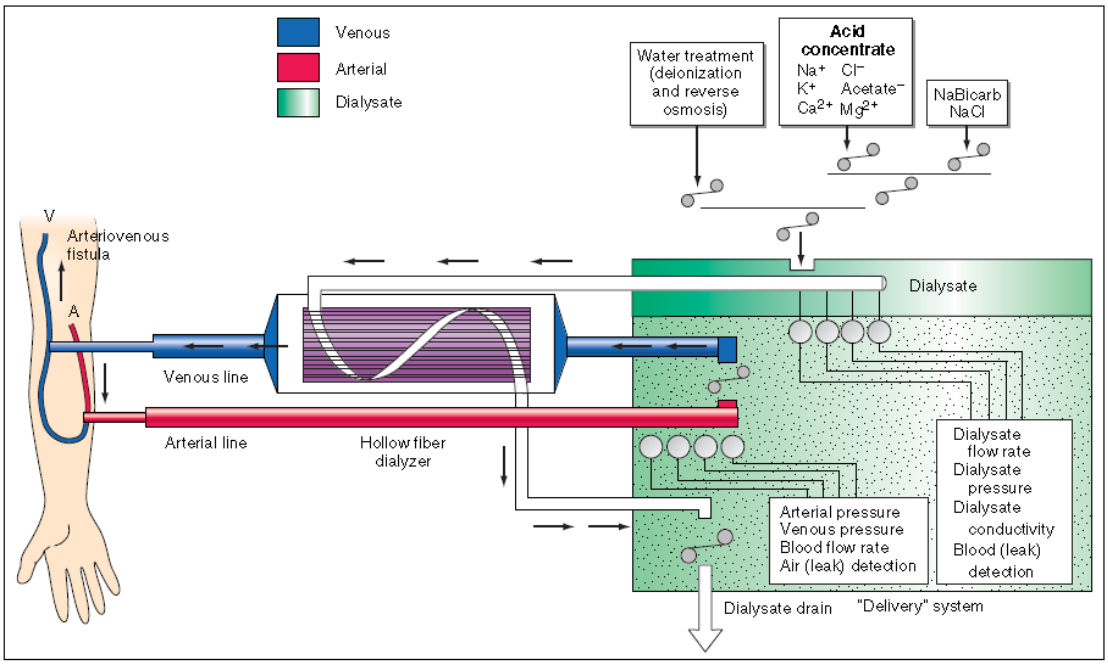

Figure 1.5 A schematic diagram of haemodialysis (Kasper and Tinsley, 2005)

The haemodialysis technique, shown schematically in Figure 1.5 consists of four major components:

- Dialyser
- Blood delivery system
- Dialysate delivery system
- Blood access

The *Dialyser* is a device that distributes thin films of blood and dialysate flowing at high velocity on either side of a semi-permeable membrane. The surface area of the membrane ranges from 0.8 to 1.2 m^2 in adult-sized devices. Dialysers are manufactured in the following configurations:

- **Hollow fibre**: these dialysers consist of a parallel bundle of thousands of small-bore tubes or hollow fibres (ca. 200 µm bore). Blood flows through the fibre lumens via manifolds located at each end of the bundle whilst dialysate passes over the exterior surface of the fibres in the opposite direction to that

of the blood flow. The early hollow fibres were made of cellulose which because of presence of free hydroxyl groups on the membrane, were not very biocompatible. But over the past two decades, switching from cellulose to substituted cellulose (e.g. cellulose acetate) and cellulosynthetic, it has been tried to bond the hydroxyl group to either acetate or tertiary amino groups, resulting in limited complement activation (more biocompatible). Nowadays, synthetic membranes such as polysulfone, polymethylmethacrylate and polyacrylonitrile membranes are used because they are more biocompatible as they do not carry these hydroxyl groups (Longo et al., 2008).

- **Flat plate**: in these dialysers blood flows between two flat sheets of membrane which are supported on grooved plastic plates. Dialysate flows within the grooves and counter current to the blood flow. These dialysers are less frequently used as their priming volume is higher than hollow fibre dialysers (100-120 ml vs. 60-90 ml). Furthermore, they are more difficult to prepare for re-use (on the same patient) in centres that have the re-use facility (more common in the United States).

Blood delivery system: this is a part of dialysis machine that runs blood through the dialyser. Blood from a vascular access site flows into the extracorporeal tubing and is monitored for the presence of air bubbles, particulate matter, pre/post dialyser pressure and temperature (in some advanced machines an on-line haematocrit measurement is available). A roller-type blood pump circulates blood from the access site, through the dialyser and back to the access site at flow rates ranging from 250-500 ml/min.

Dialysate delivery system: this component dilutes dialysate concentrate with decalcified and purified water (reverse osmosis) and passes this fluid through the dialyser and discards the used solution at the end. The conductivity, temperature and pressure balance of this fluid is constantly monitored by several sensors on-line. A dialysate flow of 250-700 ml/min (based on the machine) can be set and a negative

pressure is applied to help the user to define how much plasma water is needed to be ultrafiltered during a treatment session.

Blood access is another essential element of haemodialysis treatment which provides an adequate blood supply for the extracorporeal circuit. Blood access can be an arteriovenous fistula, arteriovenous graft or catheter:

- An AV fistula is created subcutaneously by anastomosis of an artery to a nearby vein (usually in the forearm) which results in arterialisation of the vein. This means that the modified vein is supplied by blood flow from an artery and is more accessible to cannulate as veins are more superficial than arteries.
- An AV graft consists of a synthetic vascular graft material, such as polytetrafluoroethylene, which is surgically placed between an artery and a vein.
- Catheters are easier to place in and can be used relatively soon after insertion, while fistula and grafts require a maturation period of between 6-8 weeks before use.

1.3.5 IHD- Intermittent haemodialysis

Intermittent haemodialysis is typically administered by conventional dialysis machine which has been used for chronic renal failure patients since the early days of using dialysis. Each treatment session takes between 3-5 h with a blood flow rate of 300-400 ml/min and dialysate flow rate of 500-800 ml/min (Fieghen et al., 2009). The high solute clearance and fluid volume removal of this technique has advantages of rapid correction of electrolyte imbalances (e.g. hyperkalaemia) or removal of drugs and other substance intoxication. However the main disadvantage of IHD is the risk of systemic hypotension which is caused by the rapid fluid and electrolyte removal. Eventually, despite all the efforts of correction methods (profiling sodium,

increasing the dialysate concentration and cooling the dialysate) hypotension can limit the efficiency of IHD and lead to poor solute clearance (O'Reilly and Tolwani, 2005). Therefore, maintaining the rate of acid-base balance and fluid balance becomes challenging within a short time of a typical 4 hour dialysis session.

1.3.6 CRRT- Continuous Renal Replacement Therapy

CRRT is aimed to apply treatment for a duration of 24 hours and requires specific setting on the machine to deliver a lower blood and dialysate flow rate, compared to IHD treatment. Unlike IHD, the blood flow rate varies from 100-200 ml/min and the dialysate flow rate of 17-40 ml/min (O'Reilly and Tolwani, 2005). CRRT may be accomplished through the following different techniques:

Continous Haemodialysis- in CHD, dialysate fluid is passed through a dialyser at a slow rate to remove a fluid volume of 3-6 litres over an extended period of time. A typical choice of access is venous -venous (Daugirdas and Ing, 1994).

Continous Haemofiltration- in CHF a special machine runs the blood through a dialyser without using dialysis fluid where the dialyser operating purely as an ultrafiltration device. A large volume of ultrafiltrate (25-50 l/day) is replaced before (predilution method) or after dialyser (post dilution method), by infusing fluid into the blood circuit. Eventually the total volume of ultrafiltrate is calculated by adding the replacement fluid to the excess body fluid (35-55 l/day) (Daugirdas and Ing, 1994).

Continous Haemodiafiltration- CHDF is a combination of both the above methods. It means that haemodialysis is performed by running blood through a dialyser by means of a dialysis machine but with a large volume of replacement fluid used to compensate the ultrafiltered fluid. The daily volume of ultrafiltrated fluid is high in this method but in total is less than in HF (20 l/day) (Daugirdas and Ing, 1994).

1.3.7 Hybrid techniques

Sustained Low –Efficiency Dialysis - SLED which sometimes is referred as Extended Duration Dialysis employs conventional dialysis machinery but the treatment time runs between 8-12 hours. In Table 1.1 all the previously mentioned RRT modalities are compared in terms of duration of each treatment, blood/dialysate flow rates and anticoagulant requirements.

Table 1.1 Practical comparison of acute RRT modalities (Fieghen et al., 2009)

	Intermittent hemodialysis (IHD)	Sustained low efficiency dialysis (SLED)	Continuous renal replacement therapy (CRRT)
Session duration, h	3-5	8-12	24
Blood flow, ml/min	300-400	200-300	100-200
Dialysate flow, ml/min	500-800	200-350	25-40
Anticoagulation requirement	heparin or none	heparin or none	heparin or regional citrate

CHAPTER TWO

HAEMODIALYSIS TECHNOLOGY AND

INTRODUCTION TO RENAL tubule ASSIST DEVICE (RAD)

2.1 Brief History of Haemodialysis

The concept of dialysis was described for the first time, by Thomas Graham in 1854, whilst working as a Professor of Chemistry at the University of London. At the same time the physician Richard Bright was conducting research on renal failure at Guy's Hospital, London.

Graham set up a device to show how urea can pass through a membrane. He hung a bell-shaped vessel with an ox-bladder membrane inside another container filled with distilled water as shown in Figure 2.1.

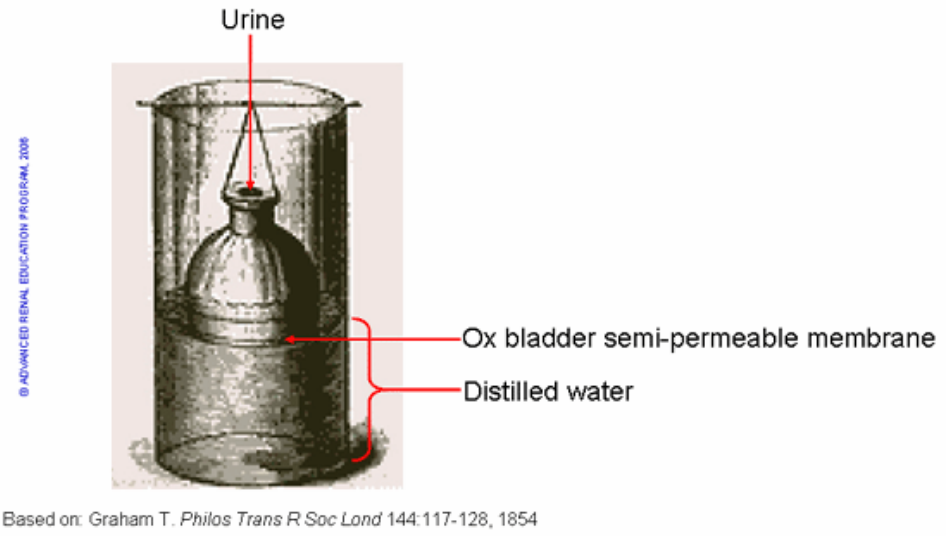

Figure 2.1 Thomas Graham's device containing a bell-shaped vessel (Diaz-Buxo, 2006)

Urine was placed in the bell-shaped vessel. After a few hours the vessel was removed and the fluid remaining in the outer container was heated. Upon evaporation of the fluid, the residue left behind was shown to contain sodium chloride and urea which are urine components. This proved that these components had passed through the membrane, a process which Graham termed dialysis. Graham in conjunction with Dr Bright proposed that dialysis would be the basis of a

treatment for renal failure but that it would take about 60 years to realise. Interestingly, and because of Graham's other pioneering work conducted in the Andersonian University (a forerunner of today's Strathclyde University) and the University of Glasgow, he is commemorated by a statue erected in George Square, Glasgow (Figure 2.2).

Figure 2.2 Statue of Thomas Graham in George Square, Glasgow (Downer, 2009)

Graham also spent a great deal of time measuring the diffusion of different elements (colloids and crystalloids) through semi-permeable membranes. He believed that in order to find a successful treatment for renal failure, toxins should be removed and the kinetics of removal through a semi-permeable membrane should be understood (Diaz-Buxo, 2006).

It was not until 1924 that the technique of dialysis was applied to a uraemic patient by Hass at the University of Giessen, Germany (Hass, 1924). Hass used a device made of collodion tubes and cannulated the radial carotid arteries and portal vein for blood supply using purified hirudin as an anticoagulant. In 1937 the first flat plate haemodialyser using cellophane membranes was developed by Thalheimer (Medicine, 2012). Arguably, the scientist who has contributed the most in management of uraemic patients with the artificial kidney is Willem (Pim) Johan

Kolff (1911-2009). During World War II, in occupied Netherlands, Kolff with assistance of an engineer developed the first clinically successful artificial kidney. In this device, shown in 2.3, a cellophane sausage tubing is helically wound on a slatted cylinder (drum) which is submerged partially in dialysis fluid. Blood is delivered via a rotating coupling to the tubing lumen and propelled through the tubing by rotation of the drum. With this very method, the life of a patient with acute renal failure was saved on 3rd Sep 1945.

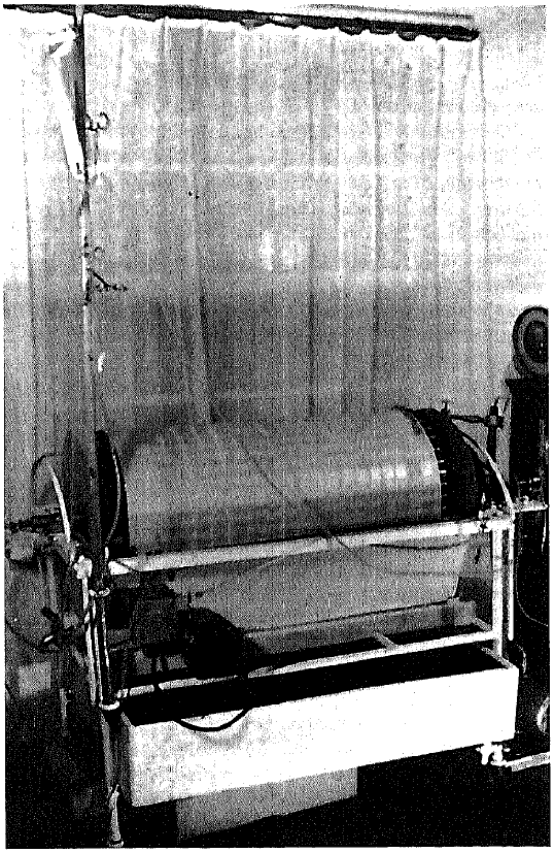

Figure 2.3 Kolff's original rotating drum artificial kidney (Drukker, 1989)

It is obvious that so many efforts of different scientists have contributed to development of what we know of a haemodialyser today. Major developments in membrane biocompatibility, dialyser geometry and function, and monitoring systems to facilitate different treatment modalities have occurred over the last four- five decades.

2.2 Conventional membranes

A dialyser consists of a box or cylinder with an inlet and outlet to allow the blood to pass through thousands of hollow fibres-tightly bound together (Figure 2.4)- or between layers of folded micro-porous sheets (Figure 2.5) (Daugirdas and Ing, 1994). It also consists of two other ports to let dialysis fluid travel around the micro fibres, through the dialyser and then into a drainage system. Dialysers are designed in a way that blood and dialysis fluid do not mix and only can exchange substances that are smaller than the pores in the membranes which is defined by the molecular weight cut-off of the membrane. In order to gain the maximum exchange between blood and dialysate, blood runs counter current to the dialysate flow.

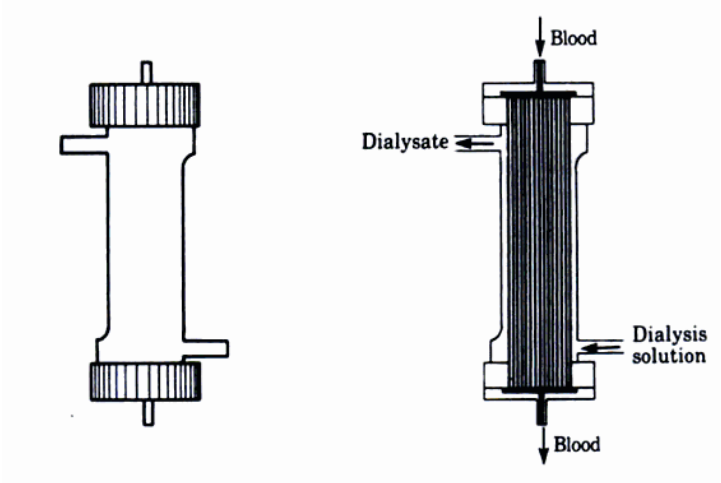

Figure 2.4 Hollow fibre dialyser (Daugirdas and Ing, 1994)

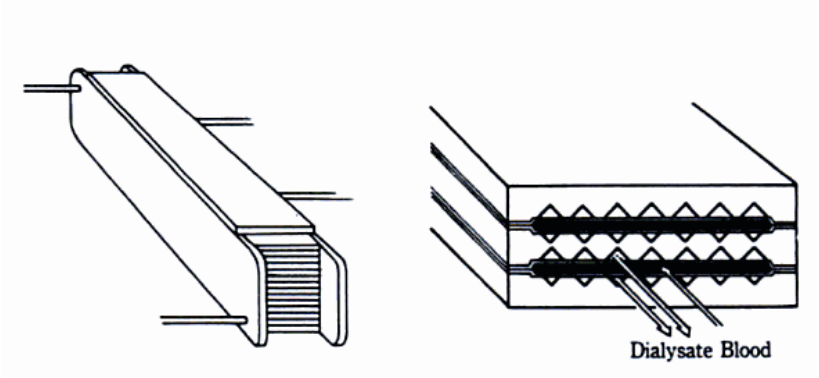

Figure 2.5 Parallel-plate dialyser (Daugirdas and Ing, 1994)

As explained earlier, many different materials have been used since haemodialysis became practical in use. Among the membrane materials listed in Table 2.2, synthetic membranes are the most biocompatible and therefore more popular than the others mentioned.

The principal of transfer of a solute through a semi-permeable membrane in a dialyser is based on the solute concentration across the membrane. For example if the patient's blood K^+ is 5.6mmol/l and the K^+ concentration in the dialysate is 2.0mmol/l, it means that the gradient of K^+ is higher on the blood side (Figure 2.6) and K^+ ions will be transported through the membrane's pores from blood side to dialysate side.

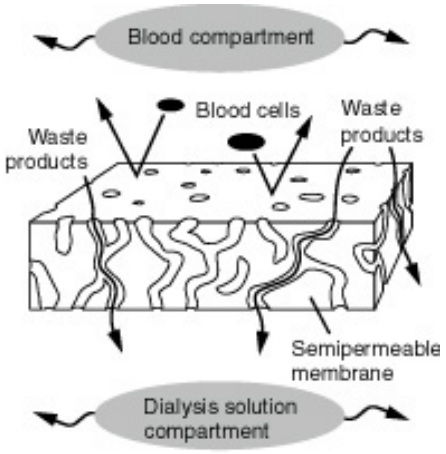

Figure 2.6 The cross section of a dialyser membrane (National Kidney and Urologic Diseases, 2012)

This results in reducing the K^+ concentration in the patient's blood over the treatment time. The whole process depends on many different factors such as blood/dialysate flow rate, membrane solute permeability and surface area, and of course treatment time. Furthermore, the higher the blood and dialyser flow rates, the greater the solute transfer in general.

The permeability of a membrane to solute and water can be altered by the thickness of the membrane and the membrane pore size. The ability of a dialyser to remove small molecular weight molecules such as "urea" relies on a constant called "the dialyser mass transfer area" coefficient or *KoA* which is measured in ml/min. A

normal range of *KoA* for a dialyser to remove urea is 500-700 ml per minute *in vitro* but this will be lower *in vivo*. For given operating conditions, the higher the surface area, the higher the transfer rate but this comes with a risk of increasing the blood priming volume which depends on the patient's body size and illness status.

The dialysis membrane can be made of different materials (Table 2.1) that brings different characteristics of blood reactions to these contact surfaces. They can be made of *Cellulose*, *Modified Substituted Cellulose* or *Synthetic materials*.

Cellulose membranes are made from processed cotton and can cause complement and leukocyte activation whereas Modified Cellulose has different chemicals substituted for the hydroxyl groups on native cellulose. They can be low or high flux membranes such as; cellulose triacetate (biocompatible) or cellulose acetate (incompatible). Synthetic membranes are not cellulose based, the materials used in the structure of these membranes are polymer based and can also be low or high flux which both are biocompatible. There are some data suggesting that limiting the effect of haemodialysis on increasing the level of oxidant substances and potentially harmful cytokines can improve the biocompatibility of the membranes (Daugirdas and Ing, 1994). For example, vitamin E-coated membrane (e.g. exebrain) can cause less oxygen free radical formation and be even more biocompatible, or some modified membranes (e.g. AN69ST) can bind heparin and reduce the total dosage needed to prevent any clotting in the circuit (Levy et al., 2004).

Table 2.1 Membrane materials (Levy et al., 2004)

	Material	***alternative names***
Cellulose	Cuprammonium rayon Cuprammonium cellulose Regenerated cellulose	Cuprophan
Modified or substituted cellulose	Cellulosynthetic Cellulose acetate Cellulose diacetate Cellulose triacetate Cellulose hydrate	Hemphan Dicea, Diaphan
Synthetic	Polysulfone Polyacrylnitrile Polymethylmethacrylate Polyamide Polycarbonate	Biosulfane, PS, helixone PAN, AN69, SPAN PMMA Polyflux Gambrane

Biocompatibility of a membrane as it is described in the text books:

> "*A biocompatible membrane elicits the least inflammatory response in patients exposed to if it does not cause complement, kallikrein*[4] *or cellular activation, has minimal interactions with proteins, and is not thrombogenic*[5]*"*(Levy et al., 2004).

These inflammatory responses, induced by different components of blood, are shown in Table 2.2

[4] The activation of killikrein-kinin system is an important factor in pathophysiology of Hypersensitivity Reactions (HRS) during haemodialysis which is a powerful proinflematory peptid. The activation of this system depends, not only on the nature of the dialysis membrane but also on the control of different physical and chemical factors. RENAUZ, J. L., THOMAS, M., CROST, T., LOUGHRAIEB, N. & VANTARD, G. (1999) Activation of the kallikrein - kinin system in haemodialysis: Role of membrane electronegativity, blood dilution and pH. *Kidney international,* 55**,** 1097-1104.

[5] low thrombin generation and release of platelet factor 4

Table 2.2 Biological response induced by interactions with dialysis membrane (Levy et al., 2004)

Blood component	*Biological response*
Complement	Alternate pathway activation Anaphylotoxin production
Coagulation system	Factor XII activation Intrinsic pathway activation
Cytokines	Some have increased levels
Erythrocyte	Haemolysis rarely
Neutrophils	Leukopenia Increase adhesion molecule expression Degranulation Release of reactive oxygen
Lymphocytes	Activation Impaired T-cell proliferation
Monocytes	Increased interlukin-1 Decreased responsiveness
Platelets	Activation Increased adhesion Thrombocytopenia Increased Factor IV and ADP release

Reaction to membranes is not always due to the type of material used but can also be caused by sterilisation methods and substances used in these methods, complement activation or even unknown mechanisms. These reactions can be called “first use” reactions or can be known as “re-use” reactions as may happen after the first time used or due to several usages. The severity of these reactions varies from mild headache, wheeze and nausea, collapse or even more fatal reactions like cardiac arrest. In 2002 a number of patients died after using Baxter Althane (cellulose acetate) dialysers. The cause of death determined later was residual *Perflurocarbon* inside the dialyser which was used to fix leaking hollow fibres, during dialyser

production. Perflurocarbon is highly hydrophobic which led to massive gas formation ending in blockage of the right side of the heart and pulmonary capillaries (Levy et al., 2004).

2.3 Renal tubule Assist Device (RAD)

One of the newest and most exciting approaches to the treatment of acute and chronic diseases is Cell Therapy. It represents a convergence of many different sciences which are rapidly progressing: stem cell biology, immunology, tissue engineering, tissue biology, biomaterials, transplantation biology, regenerative medicine and clinical research (Humes, 2005).

The basic and most successful examples of cell therapy- e.g. blood transfusion and bone marrow transplantation- have been evolved by more recent advances in cellular and molecular biology. This has led to even more potential applications of this kind of approach in treatment of disease. The bioartificial kidney clearly is one of the advances in cell therapy as a combination of living cells on polymeric scaffolds.

Research on the bioartificial kidney started with two groups, more than a decade ago: the group led by Akira Saito at the Tokai University School of Medicine, Kanagawa, Japan (Fujita et al., 2002) and another group led by Dr H. David Humes, Professor of Internal Medicine, University of Michigan USA and his team who began developing their renal tubule assist device (RAD) in the late 1990's. Other groups have also worked on the bioartificial kidney with similar bioreactor as RAD (Huijuan et al., 2007, Dong et al., 2009), trying to develop a device to clear toxins like Digoxin and another team of researchers proposed conceptually a computational design of a bioartificial nephron-on-chip (Weinberg et al., 2008).

Dr Humes and his team focused on identifying suitable adult progenitor/stem cells, designing the device and testing it in animals. The result of initial testing was published in the journal Nature Biotechnology in April 1999 (Kidney Disease, 2004).

Humes and his colleagues started to develop an extracorporeal device using a standard hemofiltration cartridge containing porcine renal tubule cells, at University of Michigan in 1999. Over 10^9 renal tubule cells were grown as confluent monolayers along the inner surface of the fibres. The non-biodegradability and pore size of the hollow fibres make them act as an immune-protective barrier as well as a scaffold for the cells. More details about the tissue engineering will be discussed in chapter Three. *In vitro* studies of this renal tubule assist device (RAD) has shown that it is capable of performing active transport of fluid and electrolytes, metabolic activities and most importantly, endocrine processes (Humes et al., 1999b).

In its initial development, the RAD was seeded with porcine renal proximal tubule cells and used along with a conventional haemofilter in a series circuit. This coupled system relies on standard ultrafiltration technology to remove small solutes and plasma water and on cell-based technology to perform the metabolic processes of the kidney. The cell-based technology relies on the ability to grow porcine proximal renal tubes in culture after harvest from 4-6 weeks old Yorkshire pigs.

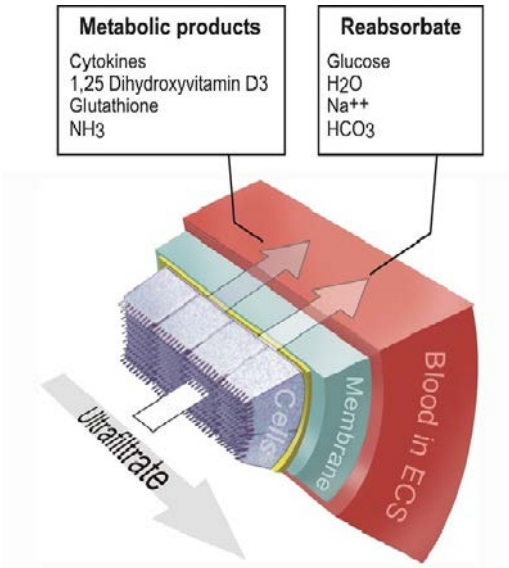

Figure 2.7 The RAD cross-section (Roger, 2005)

As shown in Figure 2.7, the cells are grown as confluent monolayers on the luminal walls of a haemofiltration cartridge containing polysulphone hollow fibres. The RAD can contain up to 2.5×10^9 cells with a membrane surface area as large as 0.7

m^2 (Peters, June 18, 1999). Successful design and fabrication of the RAD (Nikolovski et al., 1999) resulted in further experiments to establish the physiologic functionality and maintain viability of this device in an extracorporeal blood system.

A single-pass perfusion system was used to evaluate the different transport characteristics of RAD and showed a successful tissue engineering of this bioartificial device *in vivo* (Humes et al., 1999b). Further experiments demonstrated that the combination of a conventional haemofilter and RAD, successfully replaces filtration, transport, metabolic and endocrinologic functions of the kidney, even when the system is exposed to a uraemic environment in an *ex vivo* experiment (Humes et al., 1999a).

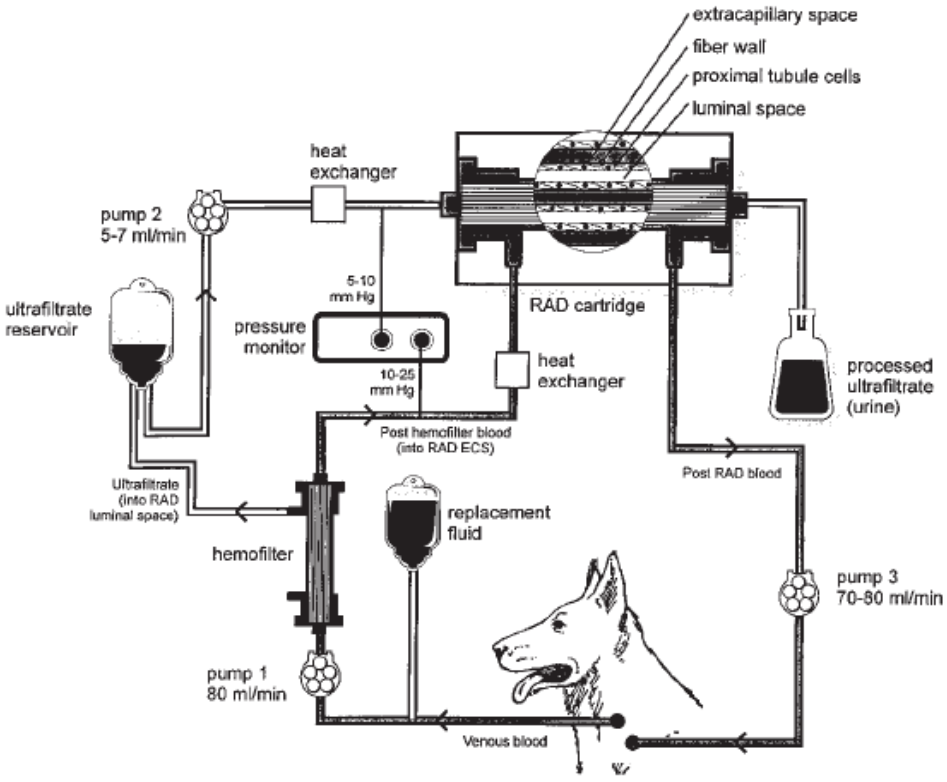

Figure 2.8 Schematic RAD in animal (Humes et al., 1999a)

Trials were conducted on a canine model (rendered uraemic by total nephrectomy) utilising the circuit shown in Figure 2.8. The set up consisted of a closed perfusion

circuit, starting from the animal's jugular vein to a conventional dialyser (haemofilter) and then to a RAD cartridge and returning to jugular vein of the dog. The initial aim of this experiment was first to study the characteristics of biochemical changes in the cultured cells. Previous to this study a mock test was conducted to find out whether the porcine cells survive in a toxic environment. A culture of porcine renal tubule cells was incubated in ultrafiltrate fluid obtained from patients with ESRD. After a week, the cells didn't show any damage and seemed well adapted in a uraemic environment (Sidorski and Peters, 1999).

The next objective was to see if the porcine renal cells can withstand the shear stress from the uraemic filtrate flowing over the cells. In the setup shown in Figure 2.8, the uraemic filtrate produced by the haemofilter enters the RAD cartridge, passes through the hollow fibre lumens and contacts the cells attached to the luminal walls. During the first run of the experiment, there was some cell loss, but decreased near to zero for the last part of the 24 hour run (Sidorski and Peters, 1999).

Finally, Humes and colleagues measured the essential elements in plasma of the dogs, before and after treatment with RAD, and compared the results with control dogs with the same condition of renal failure that were not treated. They found a significant improvement in the level of potassium and blood urea nitrogen in treated dogs, during almost 24 hours treatment. In terms of metabolic activities, from the acid and filtered ammonia levels, it was shown that the RAD cartridge was active. In addition, monitoring the level of 1,25-$(OH)_2 D_3$ (an active version of vitamin D), in repeatedly treated dogs was amazingly equal to normal dogs (Peters, June 18, 1999).

This experiment gave the scientists the idea of using cell therapy and bioreactor technology to apply the RAD in treatment of a medical problem. This was the first step towards improving the RAD so that it could be safely used on humans in large scale clinical trials, in future.

CHAPTER THREE

TISSUE ENGINEERING AND DESIGN ASPECTS OF

THE RENAL tubule ASSIST DEVICE (RAD) OF

HUMES ET AL

One aspect of cell therapy is the developing field of tissue engineering in which the techniques from the biological and engineering sciences are combined to create structures and devices to replace tissue or organ physiological function lost in acute or chronic diseases(Langer and Vacanti, 1993). In most of the applications, tissue engineering involves placing animal or human cells within an artificial matrix with cells seeded into hollow-fibre bioreactors or encapsulating members. Pilot human studies had already been started before the attempts were made to design the renal tubule assist bioreactor (RAD). For example, an extracorporeal liver-assist device was designed to replace hepatic function as a bridge to liver transplant in acute hepatitis cases (Watanabe et al., 1997). Considering the success of haemodialysis over the last four decades, it is a logical step to employ tissue engineering in treatment of acute and chronic renal failure diseases (Humes et al., 1997).

It is crucial to know that having organ function replacement in cell therapy depends on the ability to isolate and grow cells *in vitro*. These cells are supposed to be able to possess stem cell-like characteristics with a high capacity for self-renewal. They are also to be able to differentiate into cells with correct structural and functional components to perform specialised physiological tasks (Humes et al., 1999b).

Surprisingly, renal proximal tubules in adult mammals have proven more resilient for regeneration after nephrotoxic and ischemic injuries. Accordingly, a methodology has been described to isolate renal tubule cells and grow them in tissue culture (Humes et al., 1991, Humes and Cieslinski, 1992).

Dr. D. Humes and his team successfully managed to construct a single hollow fibre artificial renal tubule. This single tubule consisted of a confluent monolayer of porcine renal proximal tubule cells, which were seeded on the luminal surface of a hollow fibre. This resulted in successful engineering of a bioartificial RAD that is capable of critical differentiated transport, and improves metabolic and endocrinological functions of the kidney (MacKay et al., 1998). Renal proximal tubule segments were harvested from the kidneys of 4-6 week old Yorkshire pigs and the renal progenitor cells were selected and expanded with techniques previously

explained by Humes and colleagues (Humes and Cieslinski, 1992, Humes et al., 1991).

3.1 Cell culture

As reported in (Humes et al., 1999b), cells were grown in 100 mm Corning culture dishes with serum free, hormonally defined (DME)-Ham's F12 media, containing 2mm glutamine and specific additives listed in Table 3.1. When the cells were cultured, they were processed so that they could be ready for a passage at a concentration of 0.4×10^6 cells per ml.

Table 3.1 Cell culture medium for expansion of porcine kidney renal tubule cells (Humes et al., 1999b)

DME Ham's F12 with 2 mM glutamine plus	
Insulin	5 ug/ml
Transferrin	5 ug/ml
Epidermal growth factor	60 ng/ml
Triidothyronine	6 pM
Hydrocortisone	0.1 uM
Retinoic acid	0.1 uM
Ethanolamine	0.5 uM
Phosphorylethanolamine	0.5 uM
Prostaglandin E_1	28 nM
Dexamethasone	50 nM
Selenite	29 nM
Sodium bicarbonate	14 nM
Trace elements	
$MnCl_2 * 4\ H_20$	1×10^{-6} mM
$Na_2SiO_3 * 9\ H_20$	5×10^{-4} mM
$(NH_4)_6MO_7O_{24} * 4\ H_20$	1×10^{-6} mM
$NiSO_4 * 6\ H_20$	5×10^{-7} mM
$SnCl_2 * 2\ H_20$	5×10^{-7} mM
NH_4VO_3	5×10^{-6} mM
Final solute concentrations	
Sodium	150 mEq/liter
Potassium	4.4 mEq/liter
Chloride	137 mEq/liter
Bicarbonate	15.4 mEq/liter
pH	7.25
Glucose	315 mg/dl

3.2 RAD design and fabrication

As explained before, the efficiency of synthetic polymeric hollow fibres have been tested in previous applications of cell therapy methods. These hollow fibres are non biodegradable membranes which are water and solute permeable. The pore sizes of

these membranes are such that the membranes exclude immunoglobulins and immunocompetent cells. This means that immune-protection against non-autologous 6 and xenogeneic7 cells can be achieved (O'Neil et al., 1997).

Humes and his group used two different sizes of cartridges as a scaffold device (Humes et al., 1999b), (see Figure 3.1):

- The smaller unit: surface area of 97cm^2, a molecular weight cut-off of 50,000 Da, 128 polysulphone fibres with an inner diameter of 200µm ,a wall thickness of 40µm and a fibre length of 17 cm (Fresenius AG, Bad Hamburg, Germany)
- The larger unit: surface area of 0.4m^2, a molecular weight cut-off of 45,000 Da, 4074 polysulphone fibres with an inner diameter of 250 µm , a wall thickness of 70µm and a fibre length of 12.5 cm (Minntech Inc. Minneapolis MN, USA)

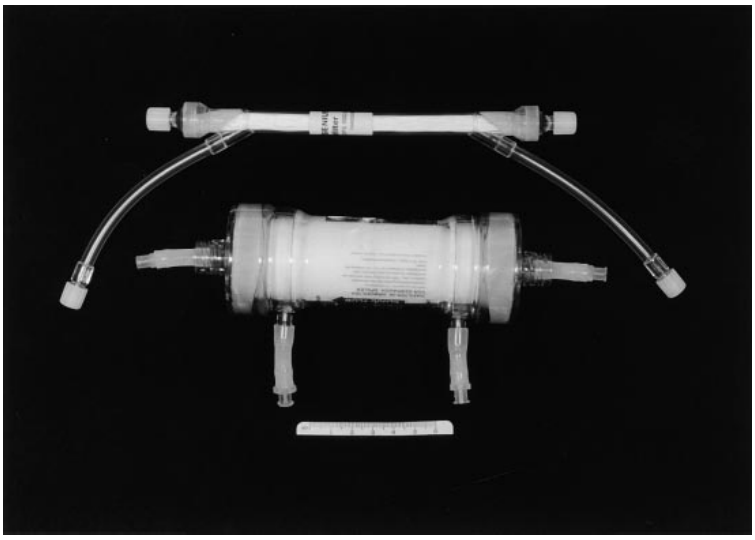

Figure 3.1 The RAD cartridges in two sizes: *a 6cm ruler is included in the picture to show the dimensional perspective of the device* (Humes et al., 1999b)

Extracellular Matrix (ECM) Different extracellular molecules were assessed for attachment of the cells to the fibre wall; collagen type I, collagen type IV, fibronectin and laminin. One of the main substances that the renal tubular basement membrane is

[6] Non-autologous: not derived from the same individual (Dorland's Medical dictionary)
[7] Xenogeneic: derived from, originating in, or being a member of another species (Dorland's Medical dictionary)

made of is laminin. Timpl et al found that laminin is best to support attachment and growth of renal cells (Timpl et al., 1979). Humes' group used a synthetic form of laminin, pronectin-L (Protein Polymer, San Diego, CA, USA) as an artificial matrix to coat the intraluminal surface of the hollow fibres. Pronectin-L is a synthetic protein which provides multiple attachment sites for the cells.

For the cell seeding process, proximal renal tubule cells (at density of 3×10^7 cells/ml) were introduced into the hollow fibres. This process was repeated for four times with 90° rotation of the cartridge and 90 minutes gaps between each cell infusion. The seeded cartridge was then connected to a bioreactor perfusion system in which the extra-capillary space (ECS) was filled with culture media and the intra-capillary space (ICS) perfused with the media.

The infusion rate was carefully increased with time, in order to let the cells adapt to the shear forces and to minimise their detachment from the hollow fibres. The infusion rate for the smaller and larger unit started initially at 2ml/min and 4ml/min, respectively. These rates were increased daily in increments of 0.4 ml/min to reach 4ml/min (smaller unit) and 6ml/min (larger unit). The intra-capillary and extra-capillary culture media solutions were changed every 2-3 days to keep enough metabolic substances for growth and viability. From the early experiments, it was shown that these cartridges needed at least 7 to 10 days of growth to reach confluency[8] level.

The number of cells in these cartridges was determined by lactate production of each unit which averaged 0.43 ± 0.01µmol/10^6 cells/24h. This resulted in an average cell number of 3.4×10^7 cells in the smaller unit and 4×10^9 cells in the larger unit. In Figure 3.2, the histological examination of different RAD cartridges shows a confluent monolayer of porcine cells lining the inner wall of the hollow fibres (Humes et al., 1999b).

[8] Confluency: in cell culture, the measure of how dense or confluent a population of cells are cultured. In a confluent culture of adherent cells, 100% of the surface of the culture vessel is occupied by cells, with no intervening space observable between cells (www.biosite.se)

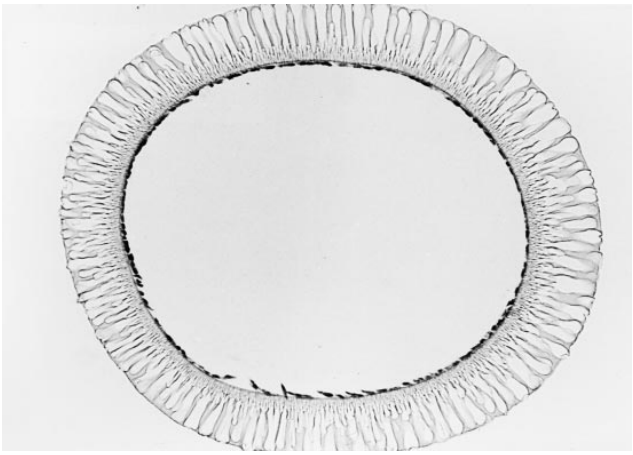

Figure 3.2 The cross section of a hollow fibre: with a confluent monolayer of porcine renal proximal tube cells along the inner surface of the fibre (a larger size of tubule is used since smaller fibres developed shear artefact when processed for histology) (Humes et al., 1999b)

Evaluation of these RAD units has shown a consistent monolayer confluency and metabolic activity for as long as 6 months after initial construction (maximal time period of performance in this experiment). It was also shown that no overgrowth of the epithelial cells, inside the tubules was observed (Humes et al., 1999b). In terms of the number of cells in a unit, it was shown that it is comparable with the number of proximal tubule cell mass in a mammalian kidney. A proximal tubule is approximately 10mm long with an outer diameter of 50µm. Humes et al describes that assuming a tubule cell as a cube with an edge of 20µm, a single proximal tubule of 10mm consists of approximately 5000 cells. Therefore, with 1×10^6 nephrons in a kidney, the proximal tubule cell number in a kidney is approximately 5×10^9 (Humes et al., 1999b).

3.3 Mathematical Modelling

One of the most important aspects of designing a bioreactor is to consider the fluid flow and its distribution. Fluid flow also has a critical role in providing nutrition for the cells and removing the metabolites away from the cells. It is crucial to have a well distributed fluid flow through the whole device.

Yvonne Moussy (Department of Mechanical Engineering - John Hopkins University) has provided a theoretical analysis of convective flow in hollow fibre modules, specifically applicable to a bioartificial haemofilter (Moussy, 2000 a). Generally, in the lumen of a hollow fibre, the existing analytical expressions describe velocity and pressure equations. In Moussy's study, the primary objective was to develop a general flow model for a hollow fibre haemofilter that would take the axial variation in the lumen pressure, shell pressure and osmotic pressure, into account and combine them together to give an accurate prediction of the filtration rate, along the hollow fibre (Figure 3.3). After providing a mathematical solution, the predictions of this model were compared to another study of simulation and mathematical modelling of CAVH haemofilter *in vitro* (Pallone and Petersen, 1988) and then used to suggest a design for an optimally functioning bioartificial haemofilter which later was applied to Dr Humes' RAD (Moussy, 2000 a). The proposed model described the velocity and pressure in the following areas:

- Solute transport in the lumen
- Lumen flow
- Shell flow
- Membrane permeation velocity

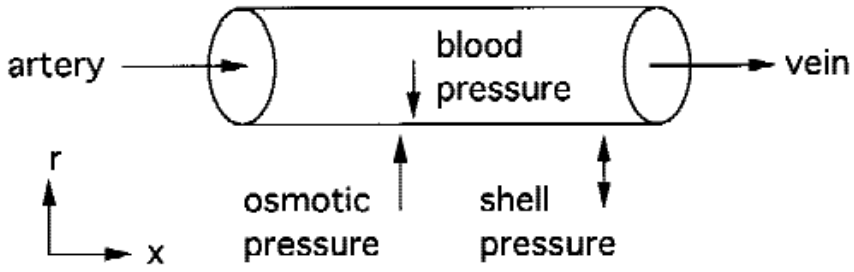

Figure 3.3 Forces that determine haemofiltration (Moussy, 2000 a)

3.3.1 Solute transport in the lumen

Since in most hollow fibres, fluid flow is predominantly in the axial direction, transport through hollow fibre is usually simplified as an axial flow problem (Brotherton and Chau, 1996). Transport across the hollow fibre membrane is both convective and diffusive. The convective transport is driven by the pressure gradient while the diffusive transport is by the concentration gradient across the membrane (Pan and Zhong, 2006). Moussy suggested that:

> "The distribution of blood cells and proteins near a membrane surface is a function of convective transport of solutes up to the membrane as a result of fluid permeation and back-diffusion of the rejected cells and proteins back to the bulk flow" the non-dimensional continuity (convection-diffusion for mass transport) equation for a solute in cylindrical coordinates is (Moussy, 2000 a):

Equation 1

$$\frac{\partial C}{\partial \tau} + U\frac{\partial C}{\partial Z} + \left(V - \frac{1}{Pe_R R}\right)\frac{\partial C}{\partial R} = \frac{1}{Pe_R}\frac{\partial^2 C}{\partial R^2}$$

And its initial and boundary conditions are:

$$C(0, R, X) = 1$$

$$\mathrm{C}(\tau, \mathrm{R}, 0) = 1$$

$$\left(\frac{\partial C}{\partial R}\right)_{\mathrm{R}=0} = 0$$

$$\left(\frac{\partial C}{\partial R}\right)_{\mathrm{R}=1} = V\,\mathrm{CP}_{\mathrm{e_R}}$$

Where: $C = {}^{c}/_{c_\circ}$ $\tau = v_{\mathrm{w}}t/r_{\mathrm{lum}}$

$V = {}^{v}/_{v_{\mathrm{w}}}$ $U = {}^{u}/_{u_\circ}$

$X = x/\mathrm{L}$ $R = r/r_{\mathrm{lum}}$ And $P_{e_R} = v_{\mathrm{w}}r_{\mathrm{lum}}/D$

where C is the solute concentration, $c_\circ$ is the bulk concentration, r_{lum} is the inner radius of the fibre, v is the radial velocity, v_{w} is the radial (wall) velocity at the inner radius, U is the dimensionless velocity, u is the axial velocity, $u_\circ$ is the velocity at the inlet centreline, t is time, L is the length of the fibre, P_{e_R} is the Peclet number in the radial direction and D is the solution diffusion coefficient.

In bioreactors more permeable membranes (high flux) are used, so axial diffusive flux can be neglected compared to the convective flux (since the axial Peclet number is much greater than 1). The initial condition (τ = 0) corresponds to a uniform concentration throughout the lumen. The inlet condition (X= 0) corresponds to a uniform solute concentration at the inlet. Symmetry condition applies at the centre of the lumen ($R = 0$) (Moussy, 2000 a).

3.3.2 Lumen flow

Moussy then suggests the following analytical solutions for a general luminal flow of a porous tube, with constant injection or suction. The velocity components for such tubes are (Moussy, 2000 a):

Equation 2

$$u = 2u_{avg}\left[1 - \frac{2v_w x}{u_{avg} r_{lum}}\right]\left[1 - \left(\frac{r}{r_{lum}}\right)^2\right]$$

Equation 3

$$v = v_{\text{w}}\left[2\left(\frac{r}{r_{\text{lum}}}\right) - \left(\frac{r}{r_{\text{lum}}}\right)^2\right]$$

And the expression for the axial pressure:

Equation 4

$$P(x) = P_\circ - \frac{8\mu u_{avg} x}{r^2{}_{lum}}\left[1 - \frac{v_w x}{u_{avg} r_{lum}}\right]$$

Where μ is the similarity variable $\mu = \left(\frac{r}{r_m}\right)^2$

3.3.3 Shell flow

To describe the shell flow (or Extracapillary space- ECS), Moussy adopted an arrangement of hollow fibres called the Krogh geometry, suggested by (Waterland et al., 1975) where the fibres are assumed to form a uniform annulus so that each fibre is at the same distance from its 6 adjacent fibres (Figure 3.4).

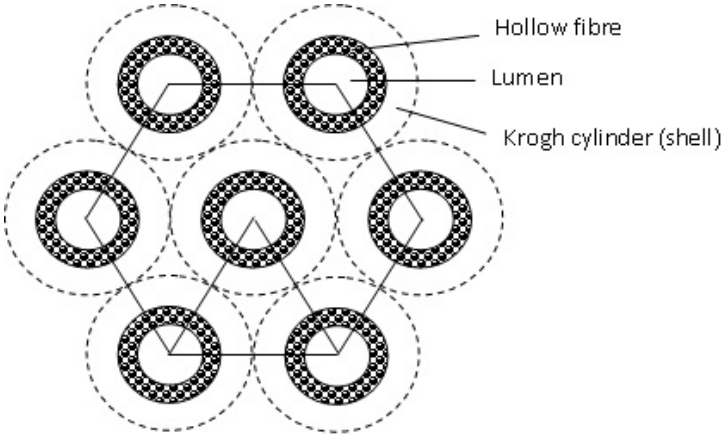

Figure 3.4 Krogh cylinder geometry of the hollow fibre bundles (Pan and Zhong, 2006)

The outer radius of the hollow fibre denoted as r_m and the radius of the shell region is referred as shell (Krogh) radius which defines the boundary between adjacent fibres and denoted as, r_s (Figure 3.5). The entire axial flow in the hollow fibre bundle is then modelled as one lumped fibre surrounded by a uniform shell cylinder. This approach assumes that all the fibres are identical, in terms of properties and internal flow, also perfectly straight and equally spaced. The interstitial spaces (triangular spaces) between the shell regions are neglected for further simplification (Pan and Zhong, 2006).

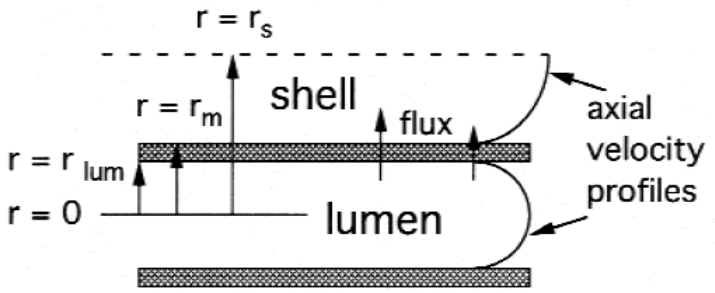

Figure 3.5 Longitudinal view of a single tube and shell arrangement (Moussy, 2000 a).

Moussy suggests this problem as an approximation of flow in an annular duct with injection at the lower boundary and symmetry conditions at the upper boundary (Figure 3.5). The assumptions in this study were:

a) The fluid is incompressible i.e. the mass density and the velocity of the fluid assumed to be constant
b) The flow was assumed to be laminar
c) The fluid flowing through the porous wall is uniform throughout

On the basis of these assumptions, for two-dimensional incompressible flow in cylindrical coordinates, a stream function $\psi(r,x)$ was defined and the velocity components suggested in axial and radial directions as follow:

3.3.3.1 Shell flow without perfusion

In a case of flow in an annular duct with injection at the lower boundary and symmetry conditions at the upper boundary:

Equation 5

$$u_s = \frac{4v_m x}{r_m} \cdot C_s \left\{ \left[\frac{r_s^2}{r_m^2} - 1 \right] + \eta - \left(\frac{r_s^2}{r_m^2} \right) [ln\,\eta + 1] \right\}$$

Equation 6

$$v_s = -\frac{2v_m}{\sqrt{\eta}} \cdot \left[C_s \left\{ \left[\frac{r_s^2}{r_m^2} - 1 \right] [\eta - 1] + \frac{1}{2}[\eta^2 - 1] \right\} - \left(\frac{r_s^2}{r_m^2} \right) \eta\, ln\,\eta - \frac{1}{2} \right]$$

Where η is the similarity variable and then obtained the pressure distribution in axial direction as:

Equation 7

$$P_s(x) = P_{so} + \frac{8\mu C_s v_m x^2}{r_m^3}$$

Where P_{so} is the shell inlet pressure. The shell pressure decreases with x as C_s has a negative value (Moussy, 2000 a).

3.3.3.2 Shell flow with perfusion

In a case of flow in an annular duct with injection at the inner radius and symmetry conditions at the outer radius of a perfused shell (ps):

Equation 8

$$u_{ps} = \left[\frac{2Q_s}{\pi r_m^2} + \frac{4v_m x}{r_m}\right] \cdot C_s \left\{\left[\frac{r_s^2}{r_m^2} - 1\right] + \eta - \left(\frac{r_s^2}{r_m^2}\right)[\ln \eta + 1]\right\}$$

Equation 9

$$v_s = -\frac{2v_m}{\sqrt{\eta}} \cdot \left[C_s \left\{\left[\frac{r_s^2}{r_m^2} - 1\right][\eta - 1] + \frac{1}{2}[\eta^2 - 1] - \left(\frac{r_s^2}{r_m^2}\right)\eta \ln \eta\right\} - \frac{1}{2}\right]$$

where Q_s is flow rate in the shell and the pressure distribution in axial direction would be (Moussy, 2000 a):

Equation 10

$$P_{ps}(x) = P_{so} + \frac{4\mu C_s}{r_m^2}\left[\frac{2Q_s x}{\pi r_m^2} + \frac{2v_m x^2}{r_m}\right]$$

3.3.4 Membrane permeation velocity

Moussy evaluated the membrane permeation velocity at the separation layer (lumen side) and provided the radial velocity at the outer radius of the fibre, v_m as:

$$v_m = v_w r_{lum}/r_m$$

The filtration rates and trans-membrane pressure (TMP) in CAVH are minimal compared to the TMP developed by pump-driven systems and the relationship between filtration rate and trans-membrane pressure remains linear. But the oncotic[9] pressure is a nonlinear function of the protein concentration at the surface. Two equations related to either albumin concentration or plasma protein concentrations were suggested:

Equation 11

$$\pi_a = 2.8C + 0.18C^2 + 0.012C^3$$

Equation 12

$$\pi_p = 2.1C + 0.16C^2 + 0.009C^3$$

Since albumin is the main protein in plasma or blood, it was taken to be representative for blood protein. Then π_a, was calculated using the value of C at the inner surface of the hollow fibre. π_x is the osmotic pressure due to protein (or oncotic pressure.

Finally, the non-dimensional convection-diffusion (Equation 1) for albumin was solved using the forward-time centred-space (FTCS) method[10]. Each axial increment

[9] is a form of osmotic pressure exerted by proteins in blood plasma that usually tends to pull water into the circulatory system. (Dorland's medical dictionary)

[10] In numerical analysis, the FTCS (Forward-Time Central-Space) method is a finite difference method used for numerically solving the heat equation and similar parabolic partial differential

was treated as a separate membrane tube with constant tube side pressure, axial velocity, shell side pressure and shell side flow rate (if any). The osmotic pressure and then the wall velocity ($v_{m)}$ were found. Next, the radial profiles for u, v and c for the lumen and for u_s, v_s in the shell were determined from Equation 2, Equation 3 and Equation 1, respectively before going to the next axial increment. The new tube side pressure, axial velocity, shell side pressure and shell side flow rate were calculated using Equation 4, Equation 2, Equation 10 and Equation 9. After the last axial increment, the solution then progresses to the next step. The predicted filtration rates from this model was compared to the filtration rates obtained from another *in vitro* study (Pallone and Petersen, 1988). The results agreed quite well with the experimental results (Moussy, 2000 a).

3.3.5 Suggestion for a model for volume flux through a monolayer of renal epithelial cells:

Although the predictions of volume transport in this model agree with the experimental results, the functionality of RAD, as a multifibre bioreactor, is limited. RAD operates as an open-shell with perfusion in both the lumen (ultrafiltrate fluid) and shell region (blood). Therefore its functionality relies on: 1) thrombus formation in the shell region and 2) if blood delivers adequate oxygen supply to the cells. In both cases, the axial flow rate in the shell area and fibre spacing has to be considered. Following the previous model of volume transport and coupling it with the experimental data, by Moussy, the results suggested that the performance of a multiple fibre bioreactor could be improved by controlling shell inlet conditions and fibre spacing. Then Moussy suggested another method on how to improve the performance of such device by providing an analysis for modelling of volume flux through the epithelial lining of the bioartificial renal tube, in a companion paper (Moussy, 2000 a).

In order to describe the trans-epithelial forces for solute and volume reabsorption in bioartificial renal tube, Moussy used a same model used for rat proximal tubule used

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equations. TANNEHILL, J. C., ANDERSON, D. A. & PLETCHER, R. H. (1997) *Computational Fluid Mechanics and Heat Transfer*, Taylor & Francis.

by Weinstein (Weinstein, 1986 a, Weinstein, 1986 b). The epithelial transport equations suggested in the model are:

Equation 13

$$J_v = J_o - L_p \sigma C$$

Equation 14

$$J_s = N + hC + J_v(1 - \sigma)C_o$$

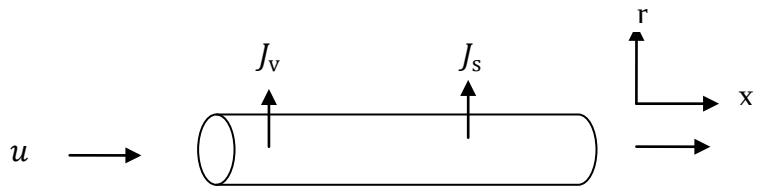


Figure 3.6 The model for volume flux: The cylinder represents as the proximal tubule, the perfusion velocity is $\boldsymbol{u}$, the reabsorptive volume and solute fluxes are $\boldsymbol{J}_v$ and $\boldsymbol{J}_s$, respectively (Moussy, 2000 b).

Where J_v is the reabsorptive volume (Figure 3.6); J_s, the solute flux; C_o, the reference osmolality;C, the osmotic difference (from lumen to bath); L_p, the epithelial water permeability; σ, the salt reflection coefficient; N, is the active transport term; and J_o, the coupled water flux. In bioartificial modelling J_o is the component of water flux which does not directly affect either transepithelial hydrostatic or osmotic forces (Moussy, 2000 b).

Weinstein (Weinstein, 1986 a) combines these equations for epithelial solute and water transport with the relation for mass conservation (Moussy, 2000 b):

Equation 15

$$-J_v = A\frac{\partial u}{\partial x}$$

Equation 16

$$-J_s - A\frac{\partial C}{\partial t} = \frac{\partial [Au(C_o + C)]}{\partial x}$$

Where: A is the area cross-section; u is the tubule fluid velocity; x is the distance along the tubule and t is time.

Substituting Equation 13into Equation 15 gives:

Equation 17

$$-\left(J_o - L_{\mathrm{p}}\sigma C\right) = A\frac{\partial u}{\partial x}$$

Equation 18

$$-J_o + L_{\mathrm{p}}\sigma C = A\frac{\partial u}{\partial x}$$

Equation 19

$$C = \left(A\frac{\partial u}{\partial x} + J_{\mathrm{o}}\right)/L_{\mathrm{p}}\sigma C$$

Then with further manipulation of equations (13), (14), (15), (16) and (19), Moussy suggests a single differential equation representing steady-state flow along proximal tube:

Equation 20

$$u\frac{\partial u}{\partial x} + k_2 u + k_1 x = u_o k_o$$

Where u_{o} is the velocity at the tubule inlet, and

Equation 21

$$k_o = \frac{L_p\sigma^2 C_o}{A} + \frac{h}{A}$$

Equation 22

$$k_1 = \frac{NL_p\sigma}{A^2} + \frac{hJ_{\mathrm{o}}}{A^2}$$

Equation 23

$$k_2 = \frac{J_{\mathrm{o}}}{A} + \frac{L_{\mathrm{p}}\sigma^2 C_{\mathrm{o}}}{A} + \frac{h}{A}$$

Moussy explains that the cross-section area A, of the bioartificial renal tube is 2 orders of magnitude greater than for the rat proximal tubule. As a result, the values for k_2, k_1 and $k_o$are much smaller than for the rat proximal tubule. This simplifies the differential equation to:

Equation 24

$$u\frac{\partial u}{\partial x} + k_2 u = u_o k_o$$

This equation was integrated and the following relationship between u and x was found:

Equation 25

$$u + (k_0 u_0/k_2)\ln(u - k_0 u_0/k_2) = u_0 + (k_0 u_0/k_2)\ln(u_0 - k_0 u_0/k_2) - k_2 x$$

Dividing the bioartificial proximal tubule into 25 increments, the axial velocity at each increment was found by using Newton's method. In order to compare the predicted volume and solute transport fluxes of this bioartificial renal tubule model and the results from the rat proximal tubule model (Weinstein, 1986 a), Equation 19 was modified. This modification was done by multiplying the ratio of their perfusion rate to account for the difference in the perfusion rate of rat and bioartificial proximal tubules. By assuming that the cells used in the bioartificial renal tubule have similar characteristics as the rat proximal tubule cells (Weinstein, 1986 a), the values of J_v and J_s were found by substituting of the calculated parameters and then it showed that results were quite similar to the findings of the rat proximal tubule study (Moussy, 2000 b).

Another approach in mathematical modelling of a hollow fibre reactor is to consider cell growth and viability in a bioreactor. Oxygen is known to have the most important role in growth and viability of live cells, therefore it is very essential to cell differentiation and function (Colton, 1995). In a hollow fibre device, the supply of oxygen is outside (ECS) of where the cells are located (ICS). The oxygen travels from the convective flow of blood through the ECS, the diffusive transport through the permeable membrane to the cells, and the flow of ultrafiltrate through the lumen.

Understanding the rate of oxygen uptake in the cultured cells could be one of the choices in the future to reach to a mathematical analysis of oxygen depletion (Humes et al., 2006). Development of such a computational method will help to design a more efficient device with the ability of assessing its effectiveness, and facilitate the study of more specific parameters and scaling up the device.

CHAPTER 4

IN VITRO AND ANIMAL *EX VIVO* STUDIES OF THE RENAL tubule ASSIST DEVICE - RAD

4.1 *in vitro* experiment

From the cell culture, the RAD's design and fabrication and also the success in achievement of such a suitable differentiated and structural characteristic of these cells, it led to several tests to assess the RAD's functional properties.

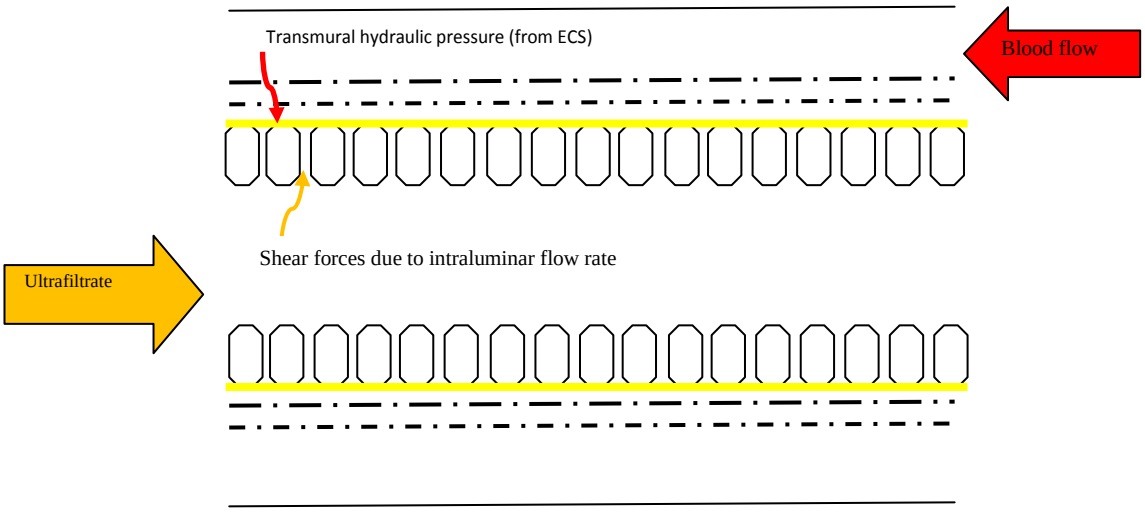


Figure 4.1 Transmural hydraulic pressures from ECS (Drawing, 2011)

To maintain cellular transport and metabolic functions of the cells within the RAD system, the cells must stay attached to the scaffold provided by the hollow fibres. They should also be able to stay viable with the provision of adequate oxygenation and nutrition while exposed to a potentially toxic uraemic environment.
The confluent tubular structure of the seeded cells in the RAD is very dependent on cellular adhesion to the scaffold. The shear forces due to the intraluminal flow of ultrafiltrate along with the transmural[11] hydraulic pressure created by the blood flow in the extracapillary space (ECS) (Figure 4.1) must be lower than the forces that result in cell detachment. On the other hand, nutrients and oxygen should be able to reach to the cells via ultrafiltrate and filtrated blood within ECS. Nikolovski *et.al* has

[11] passing or administered through an anatomical wall (http://www.merriam-webster.com)

shown that the supply of both nutrients and oxygen is more than enough to maintain cell viability and function in an extracorporeal system incorporating the RAD (Nikolovski et al., 1999). And finally, in renal failure patients, a high concentration of uraemic toxins has the potential to damage the exposed cells in a RAD unit. To find out the effects of these toxins, in a study, 35mm culture plates were used to grow porcine proximal tubule cells to confluency and exposed the cells to uraemic ultrafiltrate from three ESRD patients whom were already on dialysis treatment (Humes et al., 1999a). The ultrafiltrate samples had an average Blood-Urea-Nitrogen (BUN) and Creatinine (Cr) of 63 and 7.7 mg/dl, respectively. After 7 days there was no trace of L-lactate dehydrogenase (LDH) which is a standard method to find out if there is any cell injury (Bidlack and Lockshin, 1976).

The next step was to determine the viability and durability of a RAD unit within an extracorporeal system. The RAD was placed in series with a conventional haemofilter in an extracorporeal perfusion circuit connected to an animal with acute renal failure (Humes et al., 1999a) (Figure 2.8).

4.2 *Ex vivo* evaluation of RAD in uraemic animal model

4.2.1 Materials and method

Cell sourcing and seeding in the RAD –Porcine kidney proximal tubule cells were harvested, cultured and seeded in the manner described in CHAPTER THREE.

Animal model preparation- Dogs weighing about 25 kg were selected and underwent surgery to remove both kidneys, under anaesthesia. A double-lumen catheter was inserted into the external jugular vein to create an access for connecting to the extracorporeal system. The dogs became acutely uraemic as they were put on low protein diet before the surgery. These dogs were used 24 hours after recovery from the surgery to be put on continuous venous haemofiltration plus a RAD unit or as control group with only a conventional haemofilter.

As shown in Figure 2.8, this experimental set up consists of a conventional 0.7 m^2 polysulphone haemofilter cartridge, a RAD cartridge, standard arterial venous blood tubing and a dialysis machine. The RAD cartridge is filled with cell culture media in both intraluminar and extracapillary space (ECS). This media solution is removed by rinsing both compartments using sterile techniques and heparinised solution and once the RAD is free of air it is connected to the system. Venous blood flows from the jugular vein, to the conventional haemofilter at a flow rate of 80 ml/min with the help of a peristaltic pump. Then the ultrafiltrate which usually goes to waste in a conventional dialysis method is collected in a hanging bag. The ultrafiltrate is then infused into the intraluminar compartment of the RAD cartridge, via an infusion pump at the rate of 5-7 ml/min.

The filtered blood leaving the haemofilter is delivered into the ECS port of the RAD flows over the hollow fibres. Blood leaving the RAD is then delivered back to the animal, using another peristaltic pump at a lower rate of 70-80 ml/min to keep the balance of hydraulic pressure, throughout the system. The temperature of the whole system is monitored and controlled between 36°C-38°C with couple of heat exchangers for ultrafiltrate and filtered blood, just before entering the RAD. The processed ultrafiltrate (or urine) collected from the intraluminar port is measured and similar to haemofiltration therapy, the lost volume is compensated continuously with replacement fluid. In order to prevent any clot formation, the animal is given an initial bolus dose of heparin followed by continuous heparin infusion quite similar to a conventional dialysis.

4.2.2 Transport and metabolic measurements

To study the transport and metabolic characteristics of the RAD system, ultrafiltrate and blood samples were taken every hour from five different sites:

- Ultrafiltrate samples from pre/post RAD cartridge
- Blood samples from pre/post RAD cartridge
- Pre blood sample from haemofilter

These samples were taken four hours after starting the treatment to make sure that hydraulic pressure is stabilised through the whole circuit and re-absorption is constant, accordingly. The samples were measured for concentration of electrolytes, proteins and specifically for 1, 25-dihydroxyvitamin D3 (hormonally active form of vitamin D_3) (Humes et al., 1999a).

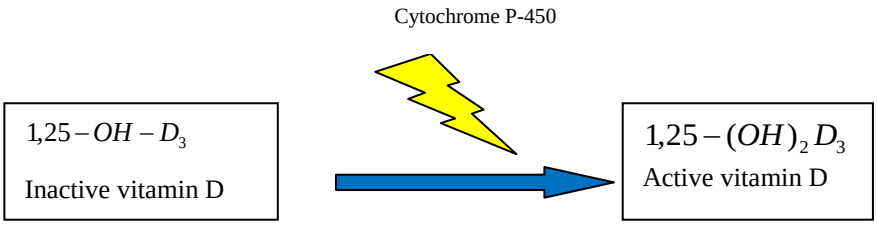
Studies have shown that the renal proximal tubule is responsible for re-absorption of 40-50% of sodium and water in the kidney and changes in hydrostatic and oncotic pressure may have effects on re-absorption (Knox et al., 1983). In Humes et al.'s study, the pressures and flow rates in the RAD treated and the control groups were adjusted so that this doesn't affect re-absorption. Accordingly, under such conditions, the tubular fluid and ultrafiltrate concentration of an element like Na should be the same on both sides of the epithelium. Therefore, the tubular fluid to ultrafiltrate concentration ratio for Na should be 1.0 and any TF:UF<1.0 suggest that a driving force exists and preferentially helps the transport of the solute, across the epithelium, which is more than the fluid transport rate and suggests an active transport process (Brenner and Rector, 1996). As the study results show, the TF:UF values for potassium, bicarbonate and glucose in the RAD group were substantially <1.0 and significantly lower than the samples taken from the control group which proves the active role of the epithelial cells in the RAD unit (Humes et al., 1999a).

In order to measure the metabolic performance of the RAD in this animal study, three critical metabolic activities of proximal tubule cells were assessed:

Ammoniagenesis: most of the nitrogen in the body is excreted as urea but the remainder (10-15%) of this nitrogen is excreted as ammonia which is the waste product of the ammoniagenic pathway (Pitts, 1945). Another role of this renal ammonia is to act as an intraluminar buffer for proton excretion and secretion (Schoolwerth, 1991). The ammonia excretion rate of the RAD group was found to increase significantly compared to the control group. Ammonia concentrations in the ultrafiltrate increased from 4-6 h towards 20-24 h period of times which correlated with the changes in luminal fluid's pH values entering and exiting the RAD.

Glutathione[12] *process*: glutathione (GSH) is an antioxidant with a critical role in defence mechanism which is freely filtered in glomerulus filtration and then reabsorbed almost fully by the proximal tubule cells. Glutathion is normally broken down into amino acids, in a normal kidney and then re-synthesised and transported across the baso-lateral membrane and back in to the cell. Because of this metabolic function of kidney, the concentration of Glutathione reduces along the proximal tube. Therefore, it is expected to have the same effect in the RAD. The fractional reabsorption rate for glutathione in the RAD-treated group found to be almost 60% ±4% (Humes et al., 1999a).

Vitamin D process: The other important role of the kidney is to produce vitamin D, among the other roles of an endocrine organ. The process of producing active vitamin D starts in the renal proximal tubule. The most active form of Vitamin D is 1,25-dihydroxivitamin D_3 ($1,25\text{-}[OH]_2 D_3$). The enzyme responsible for the conversion of inactive form of Vitamin D to the active form is cytochrome P-450 which is located in the mitochondrial membrane of proximal tube cells.



In patients with renal failure, the loss of this activity can cause a lower level of $1,25-(OH)_2-VitD_3$. Therefore, any increase in the level of $1,25-(OH)_2-VitD_3$ in patients with RAD, indicates the hormonal activity in the treatment. In the animal study, Humes et al. showed that the serum level of $1,25\text{-}[OH]_2 D_3$ of the uraemic animals stayed low before treatment with the RAD. But in the RAD-treated group the level of $1,25\text{-}[OH]_2 D_3$, significantly increased to a level close to that of the normal range, after 18-24 hours treatment (Humes et al., 1999a).

[12] Glutathione (GSH) is the reduced form of Glutathione, rather than that the oxidized form (GSSG) - Wikipedia

4.2.3 Summary

This section reviewed some of the studies to determine whether the RAD maintains differentiated renal functionality, the way it did *in vitro*, as well as whether it keeps these qualities while applied in an extracorporeal haemoperfusion circuit in an acutely uraemic animal. By evaluating the capacity of the RAD to carry out its role as a bioartificial kidney replacement, the results have shown that because of the RAD's abilities to replace many of metabolic process losses in renal failure, this form of cell therapy can optimize current therapies like haemodialysis and haemofiltration (Humes et al., 2002b).

CHAPTER FIVE

INITIAL PHASE I/II |CLINICAL TRIALS OF THE RENAL tubule ASSIST DEVICE CONTAINING HUMAN RENAL TUBULE CELLS

The earlier RAD devices were seeded with porcine cells. This was considered the best source of organ for xeno-transplantation because of the similarity in anatomy and physiology of such tissues to human's, as well as the pigs being easy to breed in large numbers (Cozzi and White, 1995). At the same time, some reports (Paradis et al., 1999, Le Tissier et al., 1997) raised the concern of the possibilities of porcine endogenous retroviruses (PERVs) infecting human cells and the risk of viral transmission between species using porcine cells in cell therapy devices. For this reason, more experiments were carried out to examine the biocompatibility of human renal tubule cells and also assessing the viability, durability and physiologic properties of the RAD containing human cells in the same setting as was done in acutely uraemic animals (Figure 2.8).

5.1 Cell sourcing and seeding in RAD (containing human cells)

Cell sources – unlike the RAD containing porcine cells, Dr Humes et al used human kidney cells from kidney transplant discards. These kidneys were those of incompatible donor/recipient kidneys. All the samples were obtained along with informed consent documentation for the purpose of ethical and legal considerations (Humes et al., 2002b). The time from the point that the kidney was Cross-clamped (when a clamp is placed on renal pedicle to surgically remove from body) and flushed with cold transplant solution and transferred on wet ice for renal proximal tubule isolation, took 30 hours (Humes et al., 2002b).

Cell isolation and tissue culture – The cells were isolated the same way as for the RAD with porcine cells (Humes and Cieslinski, 1992, Humes et al., 1991). After dissecting the kidneys, the cortex of the kidney was removed and minced. The minced cortex ($\leq$ 25g) was kept in a pre-warmed incubation solution, in separate flasks. The process of isolation was then carried on with cycles of DNase/collagenase digestions and the product of this stage was strained to separate the tubule cells. After this, the tubule cells were transferred to culture plates. The culture media were exchanged every 1 to 3 days and the cells were ready for passage every 3 to 7 days, depend on the growth rate (Humes et al., 2002b).

RAD fabrication with human cells – High-flux dialysers (polysulfone hollow fibres) with surface areas of 0.4, 0.7 or 1.0 m^2 were used in this study. Using either murine laminin or bovine collagen, the human renal proximal tubule cells were seeded the same way as described in Chapter Three, Sections 3.1 and 3.2. The cartridges prepared in this way, all contained approximately 10^9 cells which equals 6g of human cells. These human RADs have shown that they are capable of maintaining differentiated tubule cell activity for 6 months, in a cell incubator (Humes et al., 2002b).

In vitro experiments were carried out to compare Human RAD vs. Porcine RAD in active transport, metabolic and endocrine activity. Furthermore, a series of *in vitro* experiments were carried out in order to evaluate endotoxin-stimulated IL-8 production and secretion rates. The results showed that human renal tubule cells were comparable in all differentiated activities (Glucose active transport, ammoniagenesis and glutathione process) to cells isolated from porcine sources (Humes et al., 2002b).

As it has been described before, studies suggested that there is potential risk of viral transmission of PERVs from porcine cells to human tissue, if porcine cells are used in xeno-transplantation or extracorporeal cell therapy devices (Le Tissier et al., 1997). The Food and Drug Administration (FDA) had requested an evaluation carried out before any human trial could be initiated. A comprehensive study has been carried out on 160 patients whom have been treated with living porcine tissues up to 12 years before the study. The results didn't show any viral transmission from porcine to human cells (Paradis et al., 1999). Despite this evidence, the FDA hesitated to allow any clinical trial being performed using porcine cells, because of the unknown risks to the patients. However the FDA is conservatively evaluating the use of porcine cells for different clinical uses, case-by case (Humes et al., 2002b).

5.2 The initial trial of RAD in humans

The *in-vitro* and *ex-vivo* experiments of the RAD to determine the cell adherence and viability of the RAD system, as well as maintaining differentiated functionality in a toxic uraemic environment, were shown to be successful.

Considering all these results, the FDA allowed the researchers at The University of Michigan Health System (UMHS) to proceed an investigator-initiated *phase I/II*[13] clinical trial in patients with acute renal failure in intensive care units, under investigational new drug application (sponsored by Nephros Therapeutics Inc[14]), in 2002 (Humes et al., 2002b). The Phase I clinical trial started in 2001 and ended in September 2002. This study was carried out at two centres; The University of Michigan (UM) and Cleveland Clinic Foundation (CCF) in the USA. The data from 10 patients (five from each centre) were collected and treated as a single study (Humes et al., 2004). The preliminary objective of this study was to look at the safety of using the RAD on humans. The initial clinical result of the study on the first three treated patients was quite encouraging and demonstrated that this experimental treatment can be safely delivered for up to 24 hours (Humes et al., 2002a). The secondary objective was to assess RAD's capacity of replacing a normal kidney function by studying efficacy parameters, including Blood Urea Nitrogen (BUN), serum creatinine level (Cr) and urine output along with the effects of the RAD treatment on the cardiovascular and respiratory systems.

The phase II of the clinical trial of the RAD with human renal tubule cells started from March 2004 through December 2005 which involved 12 medical centres in the United States under corporate-sponsorship (RenaMed Biologics, Inc., Lincoln, RI) Investigational New Drug application (Laboratory of David H Humes, 2012). The results from this study showed a 50% improved chance of survival comparing to

[13] PHASE II TRIALS: Controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks.
(http://clinicaltrials.gov/ct2/info/glossary#phasel)
[14] Nephros Therapeutics Inc. Is a biotechnology spin out company of the University of Michigan

traditional treatment (CRRT). It also was well tolerated and the patients had a better recovery of kidney functions (Tumlin et al., 2008).

5.3 Clinical paper review

5.3.1 Patient selection

Patients whom were selected for the RAD therapy had to meet the following criteria: has an existing arterial line, diagnosis of ARF, APS15 score (Acute Physiologic Score) of moderate severity illness, non pregnant, more than 18 years older, no contraindication to anticoagulant and free from metastatic malignancy or severe chronic liver failure (Humes et al., 2004).

5.3.2 Methods

The RAD is attached in tandem to a Continuous Venovenous Haemofiltration (CVVH) circuit. As is shown in Figure 5.1, the RAD is connected in series to a CVVH extracorporeal pump system. When blood leaves the haemofilter (HF), it is pumped at a rate of 150 ml/min to the extracorporeal space of the RAD. The Ultrafitrate Fluid (UF) formed by the haemofilter is delivered into the luminal space of the RAD at the rate of 10 ml/min. The hydraulic pressures within the RAD are closely monitored and adjusted so that the UF could be reabsorbed and returned to the patient at a rate of 5 ml/min (the UF and blood pressures are both monitored before entering the RAD). The processed UF then leaves the RAD which is collected, measured and discarded as urine. The hydraulic pressures within the RAD are maintained by an additional pump located where the filtered blood leaves the Extracapillary Space (ECS) of the RAD and is returned to the patient's body. In order to maintain the optimal functionality of the cell compartment in the RAD, it is kept horizontal and placed in a temperature-controlled environment of approximately

[15] APS is one of several ICU scoring systems and is a measure of the severity of illness LE GALL, J. R., LEMESHOW, S. & SAULNIER, F. (1993) A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. *JAMA*, 270**,** 2957-63.

37°C throughout the procedure. To minimize the chance of clot formation in the system, heparin is delivered continuously upstream of the RAD (Humes et al., 2004).

In this study, all 10 patients were eligible to receive up to 24 hours of RAD therapy and only one RAD was used for each patient. All the patients were under close follow up of haemodynamics, blood gas values, serum biochemistry, liver functions and glucose levels. Glucose was specifically monitored during the first hour of treatment, as hypoglycaemia (glucose <50 mg/dL) was reported in the animal study. A cytokine assay on plasma was conducted and a variety of cytokines (such as interlukin (IL)-6, IL-10 and IL-8) were evaluated (Humes et al., 2004).

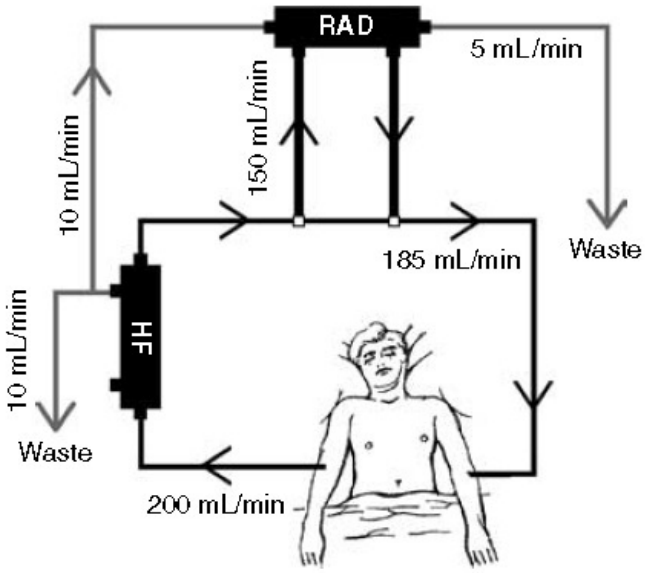


Figure 5.1 Use of the RAD in a CVVH extracorporeal circuit (Humes et al., 2004)

5.3.3 The RAD performance

The RAD compartment was checked after each use for any crack or leak which would compromise the integrity of the RAD performance. In order to evaluate a hollow fibre breakage, a sensitive fluorosphere intraluminal injection technique was used. This technique consists of microscopic assessment of the ECS of the RAD

following fluorosphere[16] perfusion into the hollow fibres. These tests showed no evidence of membrane breakage during the treatments. One of the main concerns for the RAD performance is the cell loss after each treatment. The cell loss during each treatment was examined by counting the cell numbers in hourly samples of processed UF. A range of 3×10^5 to 1.4×10^6 cells entered the UF from a total cell number in the RAD of approximately 0.5 to 1×10^9 cells (Humes et al., 2004).

The most important aspect of the RAD qualities is the ability of duplicating the transport and metabolic functional activities of a normal proximal tubule function in the kidney. These activities were assessed in all the 10 patients:

pH Due to an active transport of HCO_3 along a normal proximal renal tube, a decrease in pH happens along the epithelial monolayer (DuBose et al., 1979). Accordingly, it is expected for the RAD to behave the same way so that a decline could be seen in the pH values of Pre-RAD to post-RAD UF samples. In this study, all the patients had a pH change of either zero or net negative (Humes et al., 2004).

Glutathione (GSH) In this study, all but one patient had an average ratio of GSH post-RAD to pre-RAD concentration of <0.1. Although the changes were minor, however this means an active role of RAD in reabsorbing Glutathione (GSH) (Humes et al., 2004).

Vitamin D The analysis of all the patients' blood results revealed that only six patients had a detectable vitamin D level and they were treated more than 20 hours. Although the results showed higher levels of $1,25-(OH)_2-VitD_3$, but statistically did not reach a significant value.

Cytokine It has been previously shown that CVVH does not change the plasma cytokine levels in patients with sepsis, due to a very high level of cytokine production (De Vriese et al., 1998, Oda et al., 2010). In this study, the results from samples taken before, immediately and four hours after the treatment have shown a

[16] The Fluorospheres are polystyrene microparticles used in daily monitoring of flow cytometery. FluoroSpheres contain a mixture of microparticles of six different fluorescence intensities. (http://www.alere.co.uk)

wide variety of results. Some patients showed a modest elevation of cytokine levels, whereas others displayed more dramatic levels. However this pattern was consistent with the results of inflammatory response from septic patients undergoing CVVH. From all the patients in this study, six patients with considerably high inflammatory response were evaluated for the following cytokines: TNF-α, IL-6, IL-8, IL-10, and G-CSF. In this study, three of them appeared to be affected after the RAD therapy (Humes et al., 2004):

- IL-6 a prototypic initiator of inflammation
- IL-10 a classic anti-inflammatory compound
- G-CSF an important activator and growth factor for (polymorph-nuclear) leukocytes

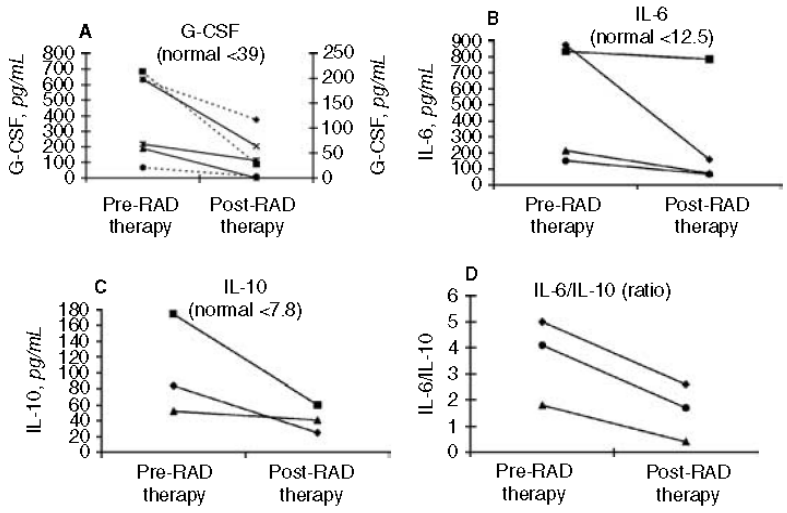


Figure 5.2 Plasma cytokine levels and ratios for excessively proinflamed patients before and after renal tubule assist device (RAD) therapy.
In (A), dashed lines refer to the left axis, and solid lines refer to the right axis (Humes et al., 2004)

As shown in (Figure 5.2), these plots are the cytokine levels in the patients, pre and post-RAD treatment who were treated for >16 hours. Plot A shows the patients who had high G-CSF level and in plot B and C, those patients who had at least three times more IL-6 and IL-10 levels than in normal. Overall, it is clear that all patients had lower levels of G-CSF, IL-6 and IL-10 levels after the RAD treatment. Despite the small number of the samples in this study, the differences in cytokines were

statistically significant by “paired *t* -test” when the values were normalized to baseline pre-treatment values. Also in three patients with highly elevated IL-6 and IL-10 levels, the results showed a significant reduction in the IL-6 to IL-10 ratio (plot D). This also means a lower IL-6 relative to I-L10 which consequently results in a less pro-inflammatory state (Humes et al., 2004).

To summarize the results of this study:

- The RAD retained its integrity, viability and metabolic functionality, throughout the treatment, up to 24 hours. There was no evidence of breakage in the RAD cartridges. The cell loss was less than 0.01% of the total approximate number of the cells to begin with.
- Metabolic functionality of the RAD was demonstrated by its effect on re-absorption of Glutathione and $1,25-(OH)_2-VitD_3$ activation. It was also shown the effects on systemic levels of cytokine before and after treatment.

Therefore the initial clinical experience of employing the RAD therapy in 10 patients with Acute Tubular Necrosis (ATN) in ICU setting has shown safety, durability, viability and also metabolic functionality for up to 24 hours.

5.4 Renal tubule Assist Device in acute renal failure

Following the successful results of using the RAD in a phase I/II trial in ARF patients, another randomised, controlled, open-label phase II investigation was approved by the FDA and started at six academic medical centres initially and then expanded to 10 centres in late 2004. In the previous study, it was demonstrated that the RAD could be incorporated in a CVVH treatment for AKI patients and safely administered for 24 hours. The study also noted an improved 30-day survival of the RAD treated patients compared to a predicted mortality from ICU scoring system of these patients (Tumlin et al., 2008). Therefore, another study seemed to be necessary to evaluate whether the RAD therapy changes the mortality rate of ARF patients undergoing CRRT at 28-days and further time points (90 and 180-days) as well as

whether the RAD has an acceptable safety measures during a treatment of up to 72 hours.

5.4.1 Results from phase II clinical research of the RAD

Fifty eight patients (age 18-80 years) with ARF who needed CRRT in ICU were randomly (2:1) selected to receive treatment:

Group CVVH + RAD: n= 40 patients

Group CRRT- alone: n= 18 patients

Both groups had similar demographic and clinical characteristics. Mean age for the RAD group was 61yr and 65 yr for the CRRT-alone. Of the total number of patients, 25 patients completed the study as planned, 31 patients died before day 180 and two patients withdrew before the study finished (one patient from each group). A higher number of patients completed the study in the RAD group (21 of 40 patients or 53%) compared to those who received CRRT-alone (4 of 18 patients or 22%). The severity of organ failure was assessed by SOFA (Sepsis-related Organ Failure Assessment) and the mean score was again almost similar in both groups (the RAD group 12.4 and the CRRT-alone group 11.5). Another ICU-severity of disease scoring system (APACHE, Acute Physiology and Chronic Health Evaluation II) showed slightly, but not significantly, higher scores in CRRT-alone group (Tumlin et al., 2008).

The primary objective of this study was to evaluate whether adding the RAD reduces all-cause mortality at day 28, 90 and 180 and improves the time to recovery of renal functions, time to ICU and hospital discharge and safety.

The RAD integrity and performance remained reasonable throughout the study. The mean time of the RAD therapy was reported as 35.9 h which ranged from 1.8 to 72.1 h. Malfunction of the RAD cartridge was only reported in 2.5% of the patients (1 out

of 40 patients). None of the cartridges were reported for excess of cell loss, leakage or noticeable haemolysis[17].

5.4.1.1 Mortality

All-cause mortality of both groups is reported in Table 5.1. Looking at the table, at day-28, of total of 58 patients, 24 patients died, 33 patients survived and one patient in the RAD group had withdrawn consent. Comparing the RAD group's mortality rate to the CRRT-alone group, 13 patients of the total 40 patients (33.3%) died, while in the CRRT-alone group, 11 patients of the total 18 patients (61.1%) had died at 28-day, though the results did not reach a statistical significance (Feng and Humes, 2008). The mortality rate reduction in the RAD group comparing to the CRRT-alone group stayed almost the same at 90-day and 180-day.

Table 5.1 Summary of all-cause mortality (Tumlin et al., 2008)

Time point	**RAD (n = 40; n [%])**			**CRRT-alone (n = 18; n [%])**			*p*
	Died	**Alive**	**Unknown**[18]	**Died**	**Alive**	**Unknown**	
28d	13 (33.3)	26 (66.7)	1	11 (61.1)	7 (38.9)	0	0.0821
90d	15 (38.5)	24 (61.5)	1	12 (66.7)	6 (33.3)	0	0.0855
180d	18 (50.0)	18 (50.0)	4	13 (76.5)	4 (23.5)	1	0.0817

[17] is the rupturing of erythrocytes (red blood cells) and the release of their contents (haemoglobin) into surrounding fluid (e.g., blood plasma) Wikipedia

[18] One patient in the RAD group withdrew consent before day 28, and three patients were alive but had their last study visit outside of the protocol-specified window of 180 ± 7 days. When these three patients are included at day 180, survival in the RAD group was 54% (21 of 39 patients). In the CRRT-alone group, one patient was lost to follow-up after day 90

In medical research, the Kaplan-Meier estimator is a statistical method which is often used to measure the fraction of patients living for a certain time after a treatment. Using this method as it is provided in Figure 5.3, survival was significantly higher in the RAD group comparing with the CRRT-alone group, through 180-day surveillance.

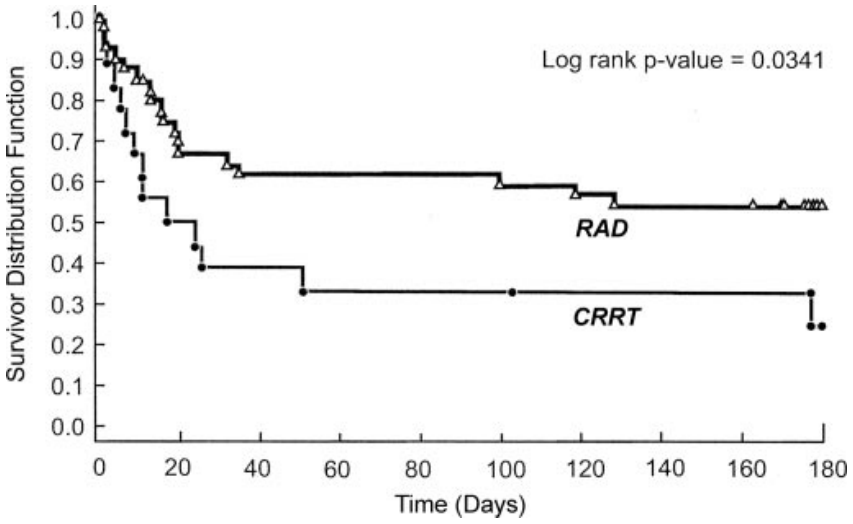


Figure 5.3 Kaplan-Meier estimates of survival between patients in the RAD and conventional CRRT groups (Tumlin et al., 2008)

The risk of death in both groups has been determined by Cox proportional- hazard regression method. Where the Kaplan-Meier method is useful for comparing two or more groups, Cox proportional-hazard regression method allows analysing the effect of several risk factors on survival (Altman, 1991). Employing Cox proportional-hazard estimator with the results in this study showed that the risk of death in the RAD group was approximately 50% lower than that of the group receiving CRRT alone (Tumlin et al., 2008).

5.4.1.2 Renal recovery

By the day 28, the recovery of renal function in the RAD group showed that 21 patients out of 40 (53%) had recovered their renal functions, 10 (25%) had died before recovery, 8 patients (20%) remained on renal support and one (3%) had

withdrawn consent. In the CRRT-alone group, a lower fraction of patients regained their renal functions (5 out of 18 patients - 28%), a higher fraction died before recovery (9 out of 18 – 50%) and almost similar proportion remained on renal support (4 out of 18 patients – 22%). At day 180, only one patient remained on renal support in each group which means 3% of the RAD group and 6% of the CRRT-alone group.

Subgroup analysis of the patient's data, such as the patient's severity of illness (SOFA), number of organ failures and presence of sepsis, were evaluated in the study. The analysis showed constantly higher survival rate in the RAD group against the CRRT-alone group, no matter how many organ failures the patients had. However in cases with more than five organ failures, the mortality rate in the RAD group was 60%, where it was 100% in the CRRT-alone group. The sepsis incidents were high in both groups (73% in the RAD group and 67% in the CRRT-alone group). But RAD therapy reduced the mortality rate in patients with sepsis, from 67% to 34% (Tumlin et al., 2008).

Table 5.2 Most common Severe Adverse Events (SAE) (Tumlin et al., 2008)

MedDRA Preferred Term	RAD (*n* = 40; *n* [%])	CRRT Alone (*n* = 18; *n* [%])
Cardiac arrest	8 (20.0)	3 (16.7)
Sepsis	3 (7.5)	4 (22.2)
Multiorgan failure	3 (7.5)	2 (11.1)
Respiratory failure	2 (5.0)	2 (11.1)
Deep vein thrombosis	2 (5.0)	1 (5.6)
ARDS	1 (2.5)	2 (11.1)
Anemia	2 (5.0)	0 (0.0)
Cardiorespiratory arrest	2 (5.0)	0 (0.0)
Colon cancer	2 (5.0)	0 (0.0)
Hematocrit decreased	2 (5.0)	0 (0.0)
Hypoxia	2 (5.0)	0 (0.0)
Hepatic failure	0 (0.0)	2 (11.1)
Hypotension	0 (0.0)	2 (11.1)

In Table 5.2, all the common Severe Adverse Events (SAE) which had happened in two or more of the patients is illustrated. The SAE reported in this table are quite common in the ICU patients with ARF receiving dialysis. However, looking at the

data, it suggests that SAE was reported 72.5% in the RAD group (29 of 40 patients) compared to 100% in CRRT-alone group (18 of 18 patients)[19] (Tumlin et al., 2008).

5.4.2 Results

The initial purpose of this randomised, multicentre study was to evaluate the effects of adding renal tubule cell therapy to conventional CVVH treatment with the goal of reduction in all-cause mortality in AKI patients in ICU at 28-day. The primary objective at 28-d mortality rate did not reach statistical significance (p= 0.08). However, as a secondary objective, the collective data at 180-day survival benefit showed 50% reduction in mortality rate which was statistically significant (p=0.038) (Tumlin et al., 2008). The treatment effects were studied where the patients were selected regardless of their age, sex and race, number of organ failure, presence of sepsis, severity or cause of disease. The results showed that a consistent survival benefit was achieved adding cell therapy to their conventional treatment. In terms of renal recovery, a higher percentage of the RAD group recovered at 28-day comparing to the CRRT-alone group (Tumlin et al., 2008). Therefore, the primary goals of the RAD therapy of improving overall patient survival and renal recovery have been achieved and at the same time patient's long-time survival was improved.

5.4.2 Clinical outcomes

The results from the first human phase I/II clinical trial showed that the RAD was sufficiently safe (Humes et al., 2004). However as it was noted in the section 5.2 (The initial trial of RAD in humans), no significant changes of parameters were observed as it should have been if the human renal proximal tubule cells had made any influence as a part of the device. There were no significant changes in pH or 1,25-dihydroxy vitamin D3 and minimum changes in Glutathione (GSH) levels,

[19] This is reported 68% and 89% in the RAD and the CRRT-alone group, respectively in the report.

comparing the post-RAD to the pre-RAD levels of the UF samples. The only considerable changes were in some of the plasma cytokine levels.

As much as the result from this study was revolutionary, the study was under heavy criticism. The results were called "underpowered" and extended to its design, implementation, analysis of the trial and reporting (Chertow and Waikar, 2008, Tasnim et al., 2010). One of the main issues was how could the study determine improvement in long-term survival when the results of short-term didn't show any significant effects, especially when the treatment didn't last more than 72hr (Chertow and Waikar, 2008). Consequently, for the same reasons, it was recommended by a group of researchers (Tumlin et al., 2008) to carry out a Phase III randomised, multicentre trial to evaluate further this method of treatment.

However, a follow-up study phase II-b was set up to bridge the result of the 53 patients study, but it was discontinued and patient recruitment was suspended following an interim analysis conducted by an independent data monitoring committee in 2006. A summary of events has been published in *Dr. Hume's Research Update* webpage (Laboratory of Dr. David H Humes, 2011). The whole clinical trial was sponsored by a company which Humes was its co-founder (RenaMed Biologics Inc.). This company was founded in 1995 and changed its name to Nephros Therapeutics Inc., in 2005. The company was restructured and changed its name to Nephrion Inc., after the suspension of the phase II-b of this study in 2007. At the moment all the enquiries about the study is redirected to its successor company CytoPherx Inc. CytoPherx Inc., as a for-profit private company is now engaged in developing and commercialising cytopheresis[20] systems for addressing inflammation-based diseases and conditions (e.g. ARF, ESRD, acute inflammation induced by coronary bypass surgery...).

In December 2011, a research agreement between Cytopherx Inc. and the University of Michigan USA authorised Lena Napolitano, professor of surgery, to direct the project over an initial two-year period at an estimated cost of $249,800 (Forrest, 2011). This company is now focused on *"A Selective Cytopheretic Inhibitory*

[20] Cytopheresis is a therapeutic technique to remove red or white blood cells or platelets from patients with certain blood disorders.(www.medicaldictionary.com)

Device" to treat the immunological dysregulation of acute and chronic renal failure (Humes et al., 2010). Dr Humes and his colleagues published the first results on this device based on the data from the phase II-b clinical trial of the RAD and the data from a subgroup treated with a sham non-cell-containing (SCD) cartridge. In this study twenty four patients were randomly selected to receive SCD treatment either with systemic heparin (n=12) or regional citrate (n=12) anticoagulation. The results showed that the group treated with citrate had a better survival (67% survival at 90-day) comparing to the group treated with heparin (25% survival at 90-day) (Humes et al., 2010). The authors of the paper claimed that this improvement is owed to the fact that the "cytopheretic" membrane of the device has caused the leukocytes to attach to its wall. They described how immune-fluorescent staining of a small number of SCD cartridges, after treatment, showed leukocyte adhesion to the outer surface of the haemofilter membranes.

5.4.3 Complications

Tissue engineering Other sources have been used in bioartificial kidney research such as; the proximal tubule-like porcine cell line LLC-PK1 (Lewis lung cancer-porcine kidney 1) (Aebischer et al., 1987, Fujita et al., 2004) and also with other animal-derived cells like Madin-Darby Canine (MDCK) (Tasnim et al., 2010). Humes used Human Primary renal proximal Tubule Cells (HPTCs) to build the RAD in clinical trials. However prior to the human trial, he had used "Porcine primary renal proximal tubule cells" for the *in vitro* and *ex vivo* studies (Humes et al., 1999a, Humes et al., 1999b). One of the criticisms on the RAD human trial is using animal cells in bioartificial kidney research where probably primary animal cells would behave and differentiate differently from primary human cells.

Another important point of the RAD tissue engineering is that the renal cells are bound to the porous membrane of the device by tight junctions so that it forms a confluent differentiated epithelium. If this doesn't happen, the functionality of the device would be compromised or absent. This has been shown for instance in the

formation of differentiated epithelia on different materials as scaffold (Tasnim et al., 2010). Furthermore, it has been found that HPTCs would not grow and survive on polysulfone (PSF) or polyvinylpyrrolidone (PVP) membrane which as it is described in Chapter Three (Tissue engineering of the RAD), it was PSF that was used in Hume's study. Cartridges made up of ECM-coated PSF/PVP are not suitable for using in bioreactors consisting HPTCs. It has been shown that the PVP component causes problems in HPTC growth and survival (Ni et al., 2010). Hollow fibres made of pure PSF appear to be hydrophobic which leads to difficulties in the adhesion of hydrophobic serum protein. Therefore to prevent protein adhesion, most of the PSF modern dialysers used in haemodialysis or haemofiltration contain hydrophilic components like PVP to improve protein adhesion (Ronco et al., 2000). Although these components improve the antifouling properties of such membranes, but leaves no room to suspect why the highly sensitive primary cells had difficulty to perform on non-adhesive surface of the RAD membrane (Tasnim et al., 2010). And in this case, even by adding a single coating of a suitable ECM (Extracellular Matrix), cell performance could not be improved (Zhang et al., 2009). Another argument which has been brought up by the critics (Tasnim et al., 2010) is that in Dr Hume's RAD trials both *ex vivo* and *in vivo* studies they used PSF/PVP hollow fibre cartridges which have been proven unsuitable for the application. Although it has been mentioned in Dr Hume's study that the ECM used as coatings of either laminin or collagen IV are indeed the most suitable matrix for HPTC, but as Zhang et al explains, it is the underlying material that influences cell performance (Zhang et al., 2009). Additionally, as it is proved that PSF/PVP is not suitable for HPTC and the fact that in most of the in *vivo* and in *vitro* trials HPTCs were not used, while in human trial the cells used were indeed HPTCs, this gives some light to the problems they experienced during the human trial (Tasnim et al., 2010).

Another important question raised by critics is whether HPTCs are the most suitable option or are there other cell types or sources that could be used instead. Due to the size of the membrane area of each bioreactor unit (0.7 -$1.0\,m^2$) it is crucial to obtain sufficient number of healthy HPTCs. At the moment these cells are prepared from non-transplantable human kidney and it is very important not to choose diseased kidneys. The limited life span and the limited sources of these cells make it more

difficult to provide a regular supply and reach a commercial level of production. In addition, primary proximal tubule cells show functional changes during different steps of tissue engineering. Furthermore, with the variety of different donors, it is difficult to achieve a minimum extensive functional characteristic in each cell batch (Zhang et al., 2009, Weiland et al., 2007).

Other complications the RAD therapy was well tolerated and most of the side effects were expected for AKI patients in ICU going under RRT. The most common side effect reported was hypotension which was most common for the first three minutes of treatment and was successfully treated with standard treatments, such as fluid replacement therapy.

Another mentioned side effect was thrombocytopenia which is again another common side effect in patients treated with extracorporeal circuit and heparin therapy. Although a relationship to the RAD therapy cannot be excluded, but no significant clinical complication related to thrombocytopenia have been reported (Tumlin et al., 2008).

There was one report of hypoglycaemia related to nonspecific adsorption of insulin to the cartridge membrane from the culture medium, which was then released when the RAD was inserted into the extracorporeal system. This was prevented by adding another flushing procedure before shipment and also careful glucose monitoring for the first 24h of the RAD therapy in addition to providing intravenous glucose supplement guidelines (Tumlin et al., 2008).

Lastly, there is another matter which is not considered as complication but might have had an influence on the results of the human trial of the RAD and is addressed in the report (Tumlin et al., 2008). The results of the report showed that renal recovery at 28-day was higher at the patients treated with RAD comparing to CRRT-alone group. Also, prior to this report an epidemiologic study (Waikar et al., 2007) had reported lower inpatient mortality rate in black patients with AKI or ARF than in white patients. The fact that the RAD group in this study had a greater number of black patients comparing to the CRRT-alone group, raises the question whether this may have had any influence on the results (Tumlin et al., 2008).

CHAPTER SIX

FUTURE DEVELOPMENTS AND CONCLUSIONS

6.1 Future developments

There is no doubt that an ideal bioartificial kidney would be the one which could provide continuous or at least prolonged treatment and not having the patients tethered to a machine. This kind of device would be either wearable or even implantable. This would improve the quality of life in these patients with spending more time away from hospitals and also minimise the high costs of dialysis treatments.

One of the major obstacles to commercialisation of the RAD is to distribute and store cell therapy devices where they are meant to be used (e.g. ICU, dialysis units...). To start with, Humes et al developed the first all-in-one culture vessel, cryostorage device and cell therapy delivery system, called Bioartificial Renal Epithelial Cell System (BRECS, see Figure 6.2). The BRECS was designed with up to 20 cell-seeded porous disks which were maintained with perfusion culture. They used adult progenitor cells from human unwanted transplant organs. The system was set up to a point when cells reached over 5×10^{6} cells/disk so that it reached a total therapeutic dose of approximately 10^{8} cells. The BREACS was then cryopreserved for storage at -80 or -120°C. Eventually, the BRECS was rapidly thawed and perfusion culture was resumed. Near pre-cryopreservation values of cell viability, metabolic activity and differentiated phenotype of functional renal cells were confirmed post-reconstitution (Laboratory of David H Humes, 2011).

6.1.1 Wearable BAK (WeBAK)

Portable artificial kidneys have been the interest of scientists due to the costs of the current treatment for the end stage renal disease (ESRD) patients. Therefore wearable artificial kidneys have been studied and put under clinical trials (Gura et al.,

2008). Nevertheless, challenges in the way of miniaturizing a cell-free artificial kidney with the current technology do not seem to be far out of reach. However this has to be more challenging when it comes to the viability and functional performance of highly sensitive epithelial cells which are facing the mechanical stress in a mobile device. While the functionality of these cells is compromised by physical damage, it might lead to reducing the role of reabsorption and causing over volume and critical condition. On the other hand, if there happened to be a breakage in the cell layer of the device, the uraemic toxins in the UF fluid would return back to the blood stream and again would cause a toxic critical situation. Other challenges would be developing new membranes to address the anticoagulation and antifouling properties of the device and also the life time of the device as exchanging the usable part involves high risk of infection and requires well-trained personnel to handle the procedure.

In 2003, Dr. Hume founded another company called Innovative BioTherapies (IBT)[21] to facilitate commercialisation of the technologies which are being developed at his academic lab at Michigan University. They are currently working to miniaturize the renal tubule component of the RAD (Figure 6.1) and combine it with sorbent dialysis (a technology for regenerating dialysis fluid) and their new cell therapy system (BREACS) (Figure 6.2) to build a wearable, continuous-acting, long-term bioartificial kidney. It is claimed that the device could be worn outside the body and it is planned to advance the product from pre-clinical trials to human trials within 3-5 years.

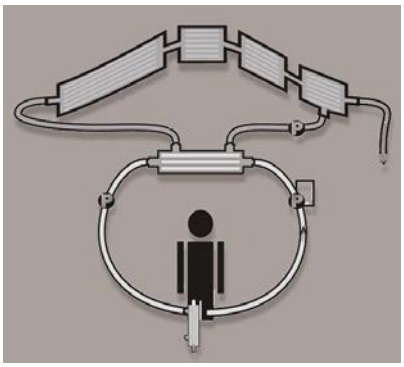


Figure 6.1 Wearable Bioartificial Kidney (WeBAK) (Laboratory of David H Humes, 2012)

[21] http://www.innbio.com/index.html

Figure 6.2 Bioartificial Renal Epithelial Cell System (BRECS) (Humes, 2010)

6.1.2 Implantable Renal tubule Assist Device (iRAD)

There are two key components involved in the implantable bioartificial kidney to be a successful device: high permeable filter which relies only on blood driving pressure for ultrafiltration and the cell bioreactor. There are also other factors involved like the package size, the device biocompatibility and life time, bioreactor cell viability and anticoagulation protocols to prevent any clot formation.

A unique technology has come to help the scientists to improve control over membrane pore characteristics and resulted in improved hydraulic permeability and molecular discrimination. MEMS (Micro-electromechanical systems) and Nanotechnology refer to study of devices and features at the cellular and molecular length scales, respectively (Fissell et al., 2007). These technologies have enabled the scientists to produce miniature, high-performance mechanical components at small unit sizes with lower cost in quantity (a familiar and simple example of MEMS devices is the nozzles of inkjet printers).

The Biomedical Microdevices Laboratory[22] University of California San Francisco (UCSF) is using MEMS technology and Nanotechnology to miniaturise a bioartificial kidney (RAD) into an implantable device the size of a cup of coffee. Shuvo Roy, associate professor of UCSF Department of Bioengineering and Therapeutic Sciences and his team of scientists are developing an implantable, self-regulating bioartificial device which is inspired by Humes' RAD which is called iRAD (Figure 6.3). This project started with Phase 1 receiving a $3.2m grant from The National Institute of Biomedical Imaging and Bioengineering (NIBIB)[23] to assess feasibility and the best approaches. This device is to be capable of all the RAD's benefits without the patient being tethered to a room sized machine and the membrane is designed to carry on filtration without needing a pump, and solely relying on the patient's blood pressure (Roy, 2011b).

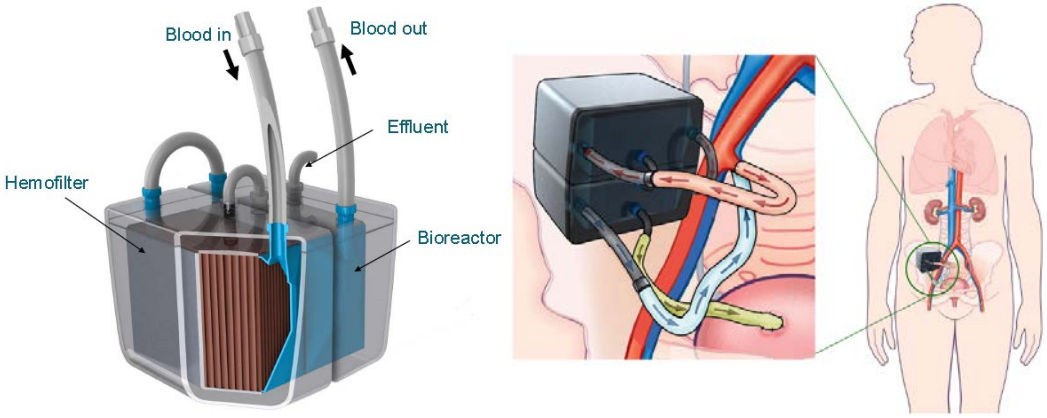


Figure 6.3 Implantable Bioartificial Kidney (iRAD) (Roy, 2011a)

This device consists of two compartments, one includes a haemofilter made up of multiple chips of Silicon Nanopore Membranes (SNM) on a wafer to perform toxin clearance (Figure 6.4) (Fissell et al., 2007).

[22] http://bts.ucsf.edu/roy/index.html
[23] http://www.nibib.nih.gov/

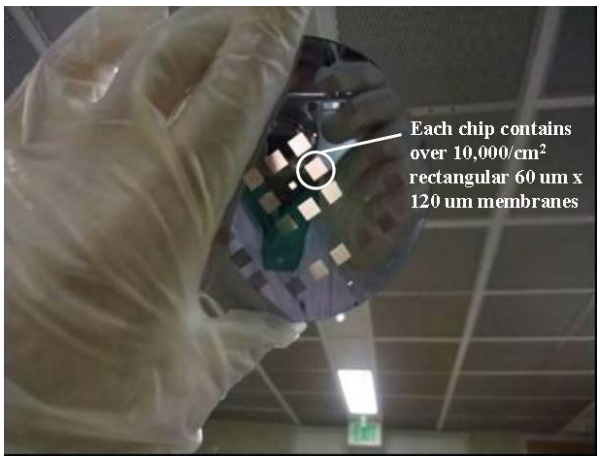


Figure 6.4 completed wafer (Roy, 2011a)

The bioreactor compartment was miniaturised by extending the use of nanotechnology to design the scaffold. With successful cell sourcing and improved storage techniques (cryopreservation of bioreactor), the bioreactor prototype was designed with new features of (Figure 6.5):

- Microchannels for controlled shear stress on cells
- Membrane for cell support and transport pathway
- Basolateral chamber for membrane support and fluid connection

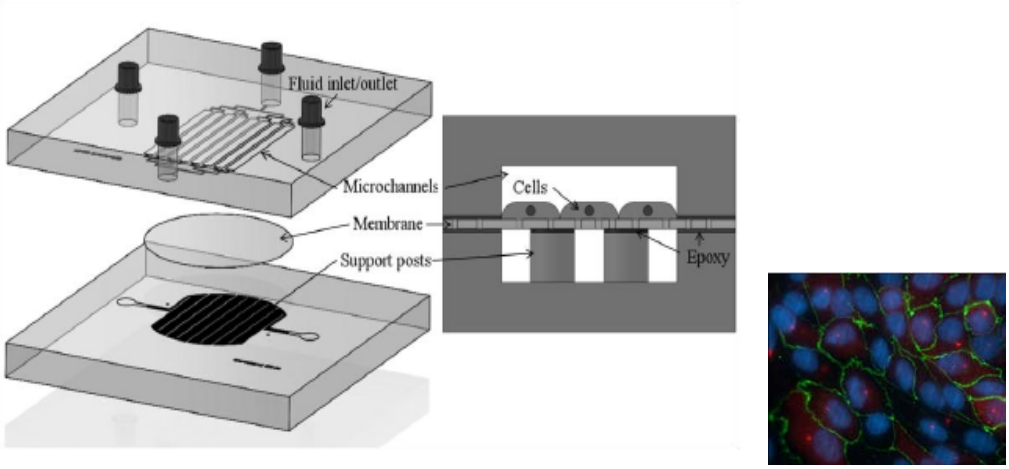


Figure 6.5 (left) Bioreactor (right) Cell Growth on Silicon membrane (Roy, 2011a)

Human renal tubule cells (HRTCs), isolated from transplant discards demonstrated significant immunoisolation and water reabsorption properties. In the first report of the current successes, Roy mentioned the key accomplishments of the Phase 1of this project since Sep 2010, in "MEPTEC[24] 9th Annual MEMS Symposium" as follows (Roy, 2011a):

- Silicon membranes:
  - high permeability
  - high selectivity
- Biocompatible coatings:
  - protein anti-fouling
  - anti-thrombogenic
  - enhanced cell isolation
- Cell sourcing:
  - harvest transplant discards
  - biopsy for autologous use
  - cryopreservation for storage
- Cell performance
  - significant immunoisolation
  - significant water reabsorption

The second phase of this project is to integrate the phase 1 successes with continuing to improve the efficiency of the device to carry out animal trials and the team is hoping to have this device ready for testing in humans within the next 10 years (Roy, 2011b).

6.2 Analysis and Conclusion

It's been more than two decades that Humes et al have been working on designing a bioartificial kidney, mimicking all of the normal functions of a kidney, to replace

[24] MEPTEC: microelectronics packaging test engineering council (http://meptec.org/)

human kidneys in renal failure patients. However other group of researchers have been involved in either improve or addressing the potential issues in the progress of developing the ideal bioartificial kidney. For example, the researchers in the Institute of Bioengineering and Nanotechnology (Singapore) have shown that HTCPs do not grow and survive on a variety of polymeric membrane materials and even coating with just an ECM on a non-compatible material does not sufficiently improve HPTC's performance (Ni et al., 2010). Another group of researchers in Tokai University School of Medicine (Japan) used human umbilical cord blood CD133(+) endothelial progenitor cells (EPCs) to evaluate the feasibility of application of EPCs and suggested that these cells would potentially be applicable in bioartificial kidney (Vu et al., 2009). And in another study, the same group of researchers investigated the development of a wearable type of bioartificial kidney and has addressed some of the technical hurdles for completion of such wearable device (Saito, 2004). Considering all the obstacles such as renal cell sources, cell performance, providing the cell containing device at clinics, costs and all other issues regarding mobile or implantable devices, it seems that a final solution needs more years of research to come.

From all the studies performed and the evidence provided about the bioartificial kidney, it seems that the most straight forward approach towards using cell therapy in RRT is the RAD as an immobile treatment for ARF patients. This is the approach that Dr. H. David Humes and his colleagues followed and the results show that more research needs to be done to overcome the current issues. One of the lessons from the initial studies is the importance of using the same source of cells in the trials as it was used in the experimental tests; otherwise it would be difficult to predict how the cells would behave under the clinical circumstances.

The issues of cell sourcing, cyto-compatibility, antifouling and anticoagulation properties as well as hydrodynamic permeability and selectivity of the membrane are among the problems that must be addressed in future studies. The recent advances from Roy and his team's work on the implantable RAD are encouraging towards solving some of these issues. He and his team are collaborating with 10 other teams including University of Michigan and specifically Dr. Humes, Cleveland clinic

(where Roy initially developed the idea), Western Reserve University, Ohio University and Penn State University to address one of the most critical medical issues of our time (Bole, 2010). And in the most recent updates of their work, the FDA announced on 9th Apr 2012 that:

> *Three renal device projects have been chosen to pilot a new regulatory approval program called Innovation Pathway 2.0, intended to bring breakthrough medical device technologies to patients faster and more efficiently. The artificial kidney project, which is targeted for clinical trials in 2017, was selected for its transformative potential in treating end stage renal disease and for its potential to benefit from early interactions with the FDA in the approval process (Bole, 2012).*

Perhaps the fast tracking of this multidisciplinary project which was targeted for 2017 would provide and facilitate a solid basis for the development of more advanced bioartificial kidney devices.

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