Fiber in Fiber (FIF) bioartificial liver device: initial design and prototyping

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ABSTRACT

A fiber in fiber (FIF) bioreactor was designed and developed for cell culture to become a bioartificial liver device. The design consists of a conventional hollow fiber cartridge as a cell culture system with the addition of a second set of hollow fibers places within the lumens of the primary set. This arrangement provides three discrete spaces (compartments). A 3D CAD model of the device was developed and used to provide the suitable arrangement of these compartments. Internal and external diameters of the commercially available hollow fibers were studied and chosen. Two configurations of the device were developed and tested. Based on a 3D CAD model, two modules of the device were constructed and studied. Experimental evaluation of small scale FIF improved device prototype were constructed and results from studied quality assurance are also presented. The results showed that the developed bioartificial liver device has an excellent oxygen transfer rate which can improve the achievement of high density cell culture.

Keywords: bioartificial, artificial, liver, fiber in fiber, prototype, bio-engineering.
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1 INTRODUCTION

Liver is the largest organ in the body, and the only one that can regenerate even if a great part of it is gone due to disease. It is one of the most important organs due to its multi-farious metabolic functions. Although, liver transplantation is the gold standard for treatment of acute and chronic liver failure, there is a need for devices that can be used as a bridge for transplantation, also more effective support therapies to either allow regeneration or as a bridge for transplantation is required. Among the various methods and approaches for the treatment of such liver failures, Bioartificial Liver devices (BALs) have proved promising and effective [1]. However, to be clinically successful, the BAL device should allow for maintenance of liver specific functions in vitro and/or to improve the achievement of high density cell culture [2]. Thus, obtaining and optimizing important design parameters before making any final prototype is very essential.

Designing and prototyping of BALs can be costly and time consuming. In this project, Computer Aided Design (CAD) software is used to develop the 3D model of a BAL device in order to enable initial assessment of the performance of different configurations of the device. A small scale FIF prototype device that mimics the functionality of the liver acinus was constructed based on the 3D CAD model for experimental evaluation.

2 LITRATURE REVIEW

The effectiveness of BAL devices in the treatment of acute liver failure depends on specific design requirements which are difficult to be fully determined due to the nature of the liver, e.g. the livers complex synthetic, metabolic, detoxification and excretion functions [3]. It is unclear yet which particular aspects of the liver function need to be replaced for the reversal of advanced hepatic encephalopathy. This presents particular problems for those groups attempting to develop bioreactor designs for liver support systems, in particular what to measure and how to optimize the design [4]. It was reported that a modular design of the BAL device would allow to change the dimensions of the device easily, i.e. scale it up or down; and to accommodate the necessary cell mass [5]. At present, the design requirements of the BAL systems to provide both quality of life and sufficient liver support duration remain unknown. For example, the cell mass and the duration of therapy likely to be required are unknown [6]. The uncertainty in these two parameters makes them important design considerations.

Moreover, certain important physical parameters in the liver, i.e. constant flow of blood, the large oxygen capacity in blood and short diffusion distances between blood and the hepatocytes, need to be considered when designing a new BAL device [7]. It is obvious that efficient mass transport will be an important parameter that heavily affects the performance of the BAL device. Therefore we decided that the design and development process are our highest priority.

Furthermore it is important that the membrane between the cell culture and the patient's perfusate should be able to 1) maximize the function of the cells by increasing the density of cultured cells; 2) promote effective exchange of substances; 3) provide cell anchorage; 4) reduce immunological hazards; 5)exhibit sieving properties; and 6) offer potential for scale-up to clinical application [8,9].

High permeability membranes allow faster diffusion of molecules and consequently a better alimentation of the cells in nutrients. However, to avoid the deterioration of the cells by the immunological system of the patient's perfusate, a limitation of the membrane pore size needs to be selected carefully [10]. This will be carefully considered in the FIF bioreactor 3D design.

Solute mass transfer in a membrane-based bioreactor not only depends on the membrane type, but also on the bioreactor geometry and the location of the compartments [11]. Inside these compartments, the phenomena responsible for mass transport would be diffusion, convection, or a combination of both processes.

HepatAssist2000 modeling analysis suggested that plasma could not transport sufficient oxygen to satisfy the demands of the cell mass in that device [12]. Recent researchers have addressed the oxygen demand of hepatocytes by incorporating integral oxygenation as in Strathclyde BAL[13], Hanover BAL[14], Goffe and Macdonald devices[15], rather than using in-series oxygenation as with VitaGen ELAD [16] and HepatAssist 2000[12].

Producing 3D constructs using CAD and prototyping technologies are not new to science. However, it has not been applied much to construct and design bioartificial livers. Computer controlled high throuput techniques can give precise and repeated 3D constructs of micrometer scales. Layer-by-layer deposition of cells using CAD was modeled by Wilson et al [17] and CAD data-manupilation techniques was also used in biomaterials [18,19].

The most challenging part in constructing a BAL device is the high density cells distribution within the hollow fibers. 3D CAD at least will help to visualize the both efficiently and uniformly of the cells distribution in the fibers. In this paper, CAD technique has been used to develop and assemble the possibilities of using commercially available hollow fibers in constructing BAL device.

In summary, there are some preliminary constraints that could be avoided without any deleterious effect on the BAL functions. Developers of BALs are staunch supporters of their own device configuration and their solutions to particular design requirements. These solutions could be different but they believe that they are correct from their experimental results. However, they all might be correct, since there are differences in hepatocytes species, flow configuration and membrane types. Therefore CAD design, theoretical considerations and literature might be helpful to avoid some mistakes when configuring a hollow fiber membrane-based liver bioreactor.

3 DESCRIPTION OF FIF BIOREACTOR

The proposed device design should be able to meet the aforementioned requirements to achieve acceptable cell viability and functions. For a bioartificial liver device, the mass transport (by diffusion or convection) from the nutrient or gas supply source to the cells serves as a key design limitation. As a result, solving mass transport limitations within BAL devices is a critical engineering challenge. As oxygen is important for cell viability and function [20-22], our goal is to develop a suitable configuration for this new FIF device to overcome the limitation of mass transport (i.e. oxygen) which occurs with other devices as mentioned by Hay [22]. The proposed device comprises a hollow fiber inside another hollow fiber bioreactor to accommodate liver cells. The configuration mimics the liver acinus since it should be able to supply oxygen at physiological partials pressures. The concept of this FIF bioreactor is different from previous multicoaxial designs of Robertson and Kim [23] and Cima [24] by the insertion of only one plasmapheresis hollow fiber inside one oxygenation hollow fiber to make one functional element of the FIF bioreactor. By enclosing parallel arrays of this element within an outer jacket, three completely separate compartments A, B and C are formed as shown in Fig. 1, where Membrane 1 separates compartments A and B whereas Membrane 2 separates compartments B and C (Fig. 2).

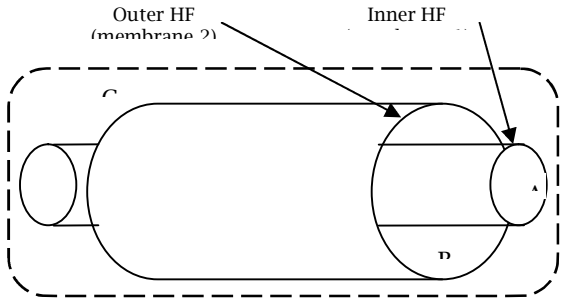

Fig. 1: Functional element of FIF bioreactor. Three separate compartments; A, B and C are defined by membrane wall of inner HF, membrane wall of outer HF and outer jacket. The outer jacket contains a number of the functional elements, which are arranged in parallel.

4 POSSIBILITIES OF THE COMPARTMENTS' USAGES

BAL devices are systems consisting of biological and artificial materials and are designed to duplicate the functions of the natural liver organ by the actions of both materials. For any BAL device, it should be able to offer a maximum function of the biological materials (cells), increase the density of cultured cells, and promote effective exchange of substances and gases as well as to reduce the immunological hazards. For these purposes, different configurations of using this FIF BAL device will be reported in this section and best one will be selected according to literature and initial in-vitro tests. As the FIF BAL device consists of three compartments, each compartment will be explained separately with the advantages and disadvantages of each choice for each compartment.

4.1 Compartment A (Comp A)

Patient's blood would be introduced into Compartment A and chemical species can be transported through membrane 1 into the compartment B either by diffusion or convection (Fig. 2). if membrane excludes all species (mid cells) of Molecular Weight above 70,000, then it acts as immunological barrier between the cells cultured in compartment B and the blood in compartment A. Hepatic failure toxins which are not protein bound toxins would diffuse into Compartment B for detoxification. Transformed species may diffuse back into Compartment A. Alternatively, if membrane 1 is configured as a plasmapheresis membrane, plasma separated from whole blood in compartment A will be convected into compartment B. Assuming cells are present in compartment B, protein-bound toxins will be detoxified and returned by back convection of plasma into Compartment A together with synthesised products. Plasma is reunited with the primary blood flow in compartment A and whole blood exits the FIF bioreactor to the patients.

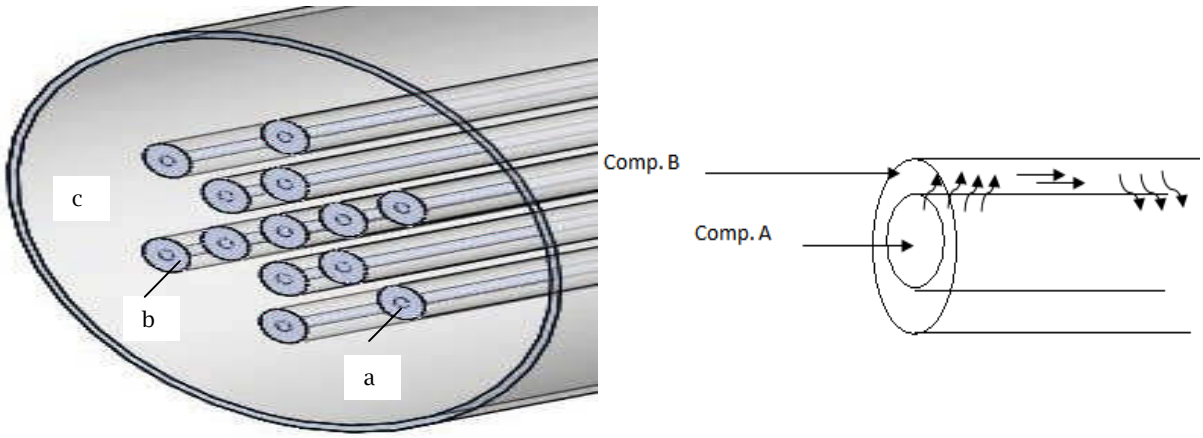

Fig. 2: 3D CAD fiber in fiber hollow fibers arrangements and the plasma flow direction. a, inner fiber which makes compartment A; b, outer fiber which makes compartment B; c, Compartment C is made from the poly-material housing.

Using the second possible way may comprise the oxygen requirements of the liver cells, which might be in Compartment B. Whereas the Oxygen requirements by the cells are met more easily with blood, plasma perfusion would require on-line oxygenation and a very high flow rate [7]. These combine to make the procedure more complicated. A further problem may be the duration over which plasma separation can be ineffective achieved before concentration polarisation renders the membrane effectiveness. Thus, the treatment time might be dictated by membrane fouling (reference).

The third compartment, i.e. compartment C, may solve the problem of oxygen delivery requirements to the cells, if it could be used as a pure gas supply space with membrane 2 configured as a gas permeable hydrophobic membrane. To get better nutrient and oxygen transport,

compartment A should not be more than 200μm in radius [25], otherwise preferable to resort to a convective flow configuration.

4.2 Compartment B

This compartment has an advantage in being interposed between the other two compartments A and C (Fig. 2). Compartment B could be used as a cell culture space as indicated before with the advantage of being able to multiply the cells much more easily than compartment A and in a better way than in compartment C. Seeding the cells in compartment C would raise biocompatibility and diffusion distance issues. The only disadvantage of using compartment B for cell culture would be the movements or the collision between the two membranes 1 and 2. This may affect the growth, life or even the function of the cells. The problem of membrane 1 and membrane 2 movements could be solved by (1) culturing the cells in a high density or (2) by using separation struts (rings, Fig. 5) at regular intervals along the hollow fiber length to maintain uniform separation.

The semipermeable hollow fiber membrane 1 would be able to protect the cultured hepatocytes from the body's immune system and alternatively able to protect the patients' blood from the toxins coming from xenogenic cells in such cells were selected. Cell to cell contact would be addressed for long-term stability of hepatic functions. For this FIF BAL device, the most important functions to be achieved would be ammonia removal and albumin synthesis as well as sufficient oxygen transfer.

4.3 Compartment C

This compartment is formed by the jacket housing and membrane 2 (Fig. 1&2). It would be used as a fluid or gas culture medium to help in keeping the hepatocytes cells alive and function for long term. This compartment also can be used as receiver of lower molecular weight substances, which have been produced by the cell metabolism. These substances will be transported by diffusion from compartment B into compartment C through a low molecular weight cut off membrane. The removal of these low molecular substances from compartment C could be supported by transport of soluble nutrients in a cell culture medium introduced into compartment C. Alternatively, this compartment might be used for circulating a gas mixture (containing O_2, N_2 and CO_2). This way of using compartment C would solve the oxygen-limiting problem suffered by most available bioartificial livers devices. If compartment C is to be used as a gas supply, membrane 2 should have the characteristics of gas-permeable membranes.

5 BIOREACTOR DESIGN

The bioreactor consists of the following parts

- Main body
- Guide plate for silicone rubber membrane only
- End collar
- End manifolds

2D CAD design model of a FIF bioreactor has been developed and parts and associated dimensions are shown in Fig. 3.

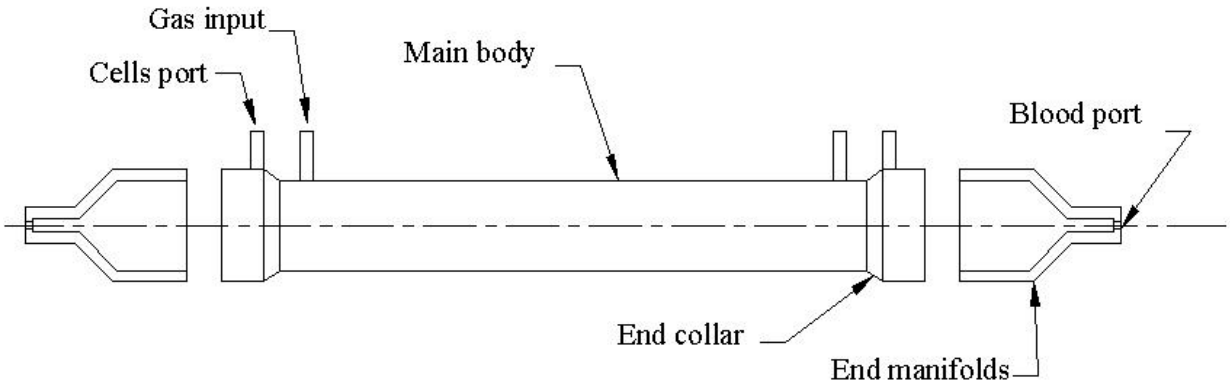

Fig. 3: 2D CAD design of component assembly of the initial FIF bioreactor design.

For symmetrical distribution of fluid (gas and liquid) through and around the fibers, a hexagonal pattern of fiber location was adapted, analogous to assumptions made by August Krogh [26] for muscle (i.e. idealised hexagonal arrangement of muscle capillaries). Sunny et al [27] studied and developed a numerical model of a hollow fiber dialyser assuming that the fibers are spaced in a hexagonal lattice and based on symmetry. This numerical model incorporates the blood, dialysate, and membrane flow in hollow fiber dialysers allowing an accurate investigation of the fluid properties and the presence and localization of backfiltration to be performed. In the FIF bioreactor, the hexagonal arrangement of the fibers is achieved by the use of guide plates (Fig. 4).

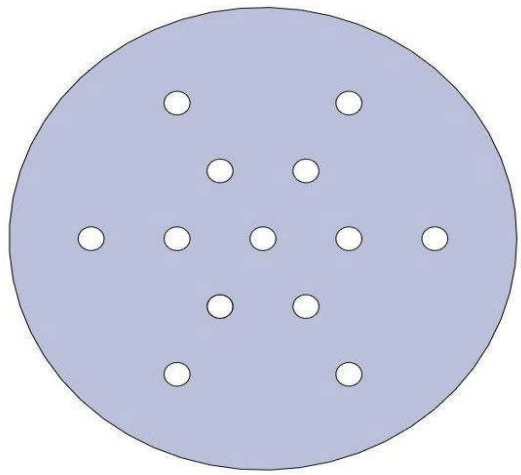

Fig. 4: 3D CAD Perforated plate with hexagonal hole-arrangement used to guide the FIF's.

The end collar also dictates the volume at the entrance and exit of the compartment. This should be much smaller than the volume occupied by the cells in the inner-fiber annulus to minimise dead volume. Construction of the initial FIF bioreactor design is done in seven stages as follows (Fig. 5):

- Cleaning procedures.
- Embedding of silicone rubber hollow fibers.

- End collar attached
- Embedding of polypropylene hollow fibers
- Partial removal of polypropylene potting resin to expose HF lumens.
- Attachment of end manifolds.
- Attachment of male Luers to inlet and outlet of compartments B and C.

Fig. 5: 3D construct of the Initial FIF bioreactor after tests.

Quality assurance was performed with the following procedures on the FIF bioreactor once the construction had been completed. The leakage of water from compartment A inlet into compartment B inlet through the polyurethane tube sheet indicated that the tube sheet was too thin to ensure complete sealing around the hollow fiber walls. Modifications to: (1) end manifolds, (2) construction method (3) holes diameter in guide plate and (4) adhesive thickness were carried out to eliminate fluid leakage and improve the overall design. Taking these results and conclusions from the fluid leakage experiments in mind, an improved FIF bioreactor was designed and constructed.

6 IMPROVED FIF BIOREACTOR

The guide plates of the FIF bioreactor are one of the important stages in designing the improved FIF bioreactor. Fig. 6 shows an MRI picture for the three compartments of Macdonald et al [15] multicentric bioreactor. It is clear that the fibers are not located co-axially.

Unlike this Macdonald design and others are having spacers to centre the inner hollow fibers and utilised hexagonal structure plates to co-axially place of the inner fibers within the outer fibers (Fig. 7). Furthermore, guide plates were machined off by non-sterile thin cutter plates with Glycerol (Sigma chemical Co., G-7757) to produce a smooth surfaced polyurethane tube sheet and patent polypropylene hollow fiber lumens.

The silicone rubber hollow fibers are located in the bioreactor as follows. The main body is clamped vertically and a guide plate placed in the recess in the upper end of the body (Fig. 7a). The hollow fibers are then threaded through the holes in the plate (Fig. 7c) and made sure that they are static. A flowable silicone rubber elastomer (RS 692-542, RS Components, Ltd) is injected onto the plate and around the fiber walls. The fibers are left for 24 hours to make sure that the elastomer has fully cured and the fibers are fixed.

The plasmapheresis membranes were attached to the silicone rubber membrane as follow, the main body with attached collars is positioned at angle of 90° and the polypropylene guide plate placed in the downward facing collar. The plasmapheresis membranes are inserted through the silicone rubber fibers and then through the holes of the guide plate Fig. 7b&c.

A 10 ml of medical grade polyurethane resin was injected through the side port of the collar until the resin surface was level with the side port. This produced an increase in tube sheet thickness comparing to the initial design. The other end was constructed in the similar fashion. The polyurethane resin (Fig. 8 shaded yellow color) along with the plasmaphersis membrane within the silicone rubber membrane is shown in Fig. 8.

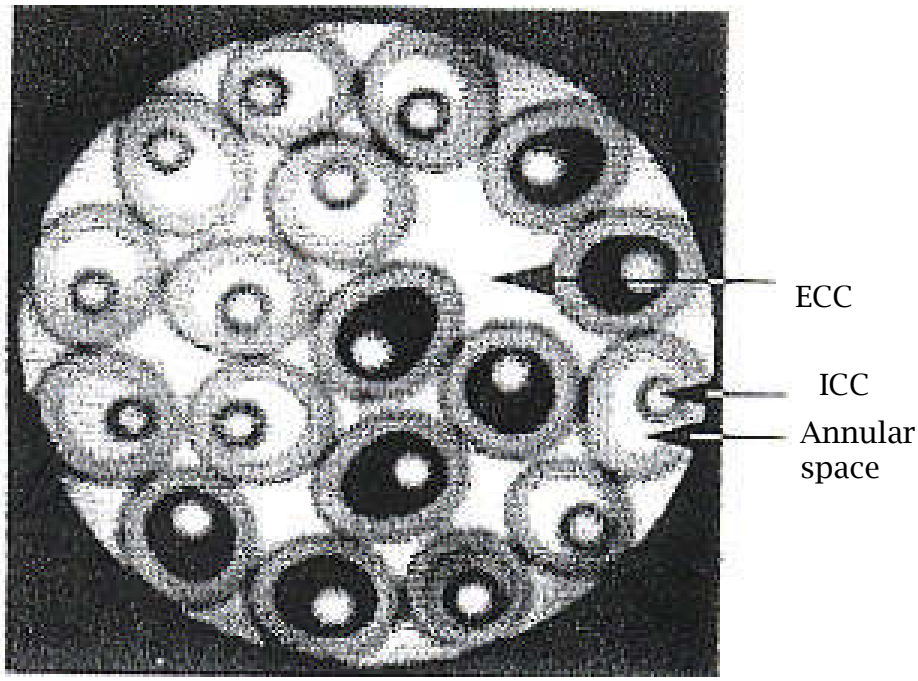

Fig. 6: MRI picture of the arrangement of the fibers of Macdonald et al design. ECC, Extra-cellular compartment; ICC, Intra-cellular compartment [15].

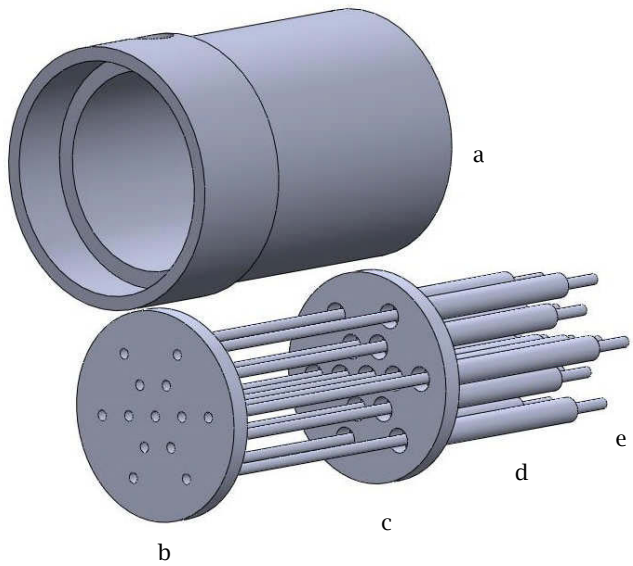

Fig. 7: Improved FIF design- polypropylene HF guide plate; Below, Silicone rubber HF guide plate.

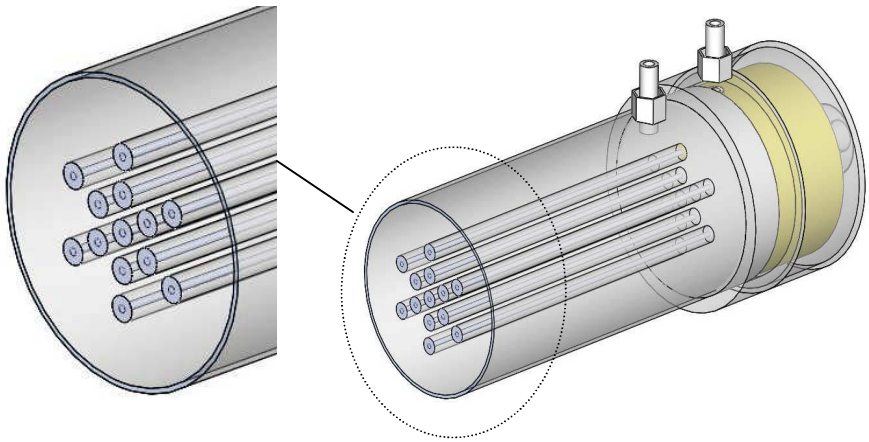

Fig. 8: the arrangement of Plasmapheresis membrane within the silicone rubber membrane, yellow color represents the polyurethane resin.

7 IMPROVED FIF BIOREACTOR QUALITY ASSURANCE

Tests were carried out to check the integrity of the seals around the compartments and to detect any leakage within the bioreactors. In these tests the bioreactor, with completed construction steps (Fig. 9), was set on a clamp at a 90° angle to avoid any bubbles at the compartments inlet. Compartment A was filled with water and sealed off to prevent any loss of water through this compartment. Distilled water was pumped (Model SB-100, JMS Corp.) through compartment B at 70ml/h, with compartment C inlet (IC) and compartment C outlet (OC) ports closed. The bioreactor and the pump were kept in an incubator at a temperature of 37°C. Bioreactors showed no bubble streams escaping from the sealing around the OB port and with more pressure we noticed that there was no water transfer through the sealing indicating that the seal is sufficient to prevent the fluid moving through.

Leakage of oxygen into Compartment A and Compartment B was investigated as well. This was done by transferring deoxygenated water into a 50 ml syringe, which was placed on a syringe pump. The water was then pumped through the bioreactor from compartment A (IA) and compartment B (IB) inlets to check the transfer in Compartment A and B respectively. Samples were taken every 2 minutes from inlet IA and IB, and compartment A outlet (OA) and compartment B outlet (OB), with 2ml syringes. At the same time a 28 % oxygen gas (balance N_2) was circulated through compartment C via inlet IC and removed at outlet OC. The pO_2 of the samples from IA, IB, OA and OB were measured by a blood/gas analyser (Rapidlab™ 248 pH/Blood Gas Analyser, Ciba Corning Diagnostics Ltd). The results of this investigation showed an increase in the pO_2 at outlet OA. This confirms the transfer of oxygen from one compartment into another by either diffusion or convection processes. This evaluation was applied to all the bioreactors separately with similar findings.

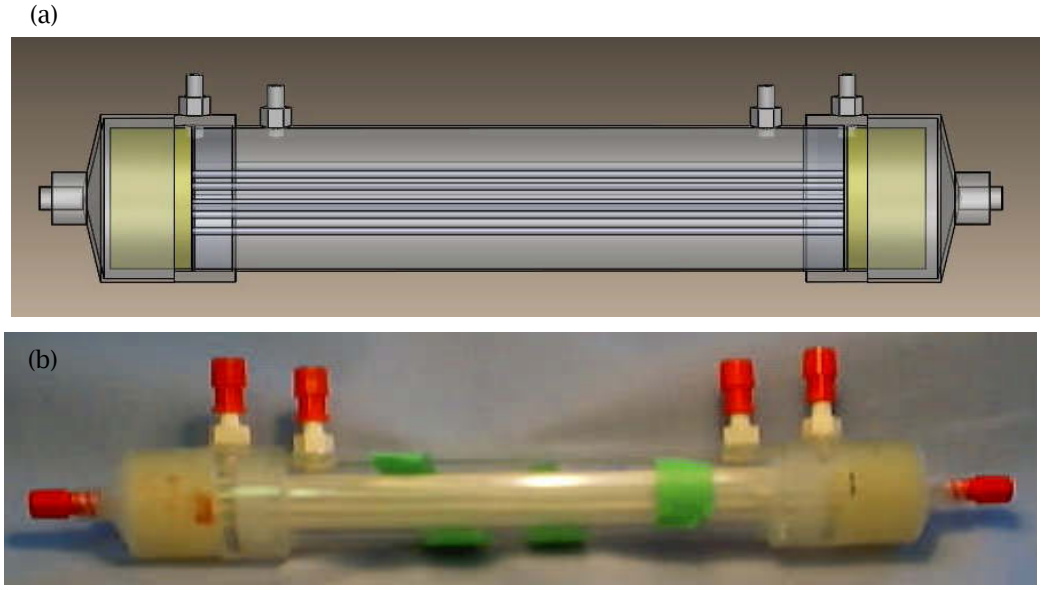

Fig. 9: (a) CAD Model and (b) 3D construct improved FIF bioreactor. Constructions and design stages are completed and ready for testing.

8 CONCLUSION

This paper presented at first a review of the designing a novel bioartificial bioreactor. This bioreactor is device for cell culture to be used as a bioartificial liver device. CAD helped to design different configurations of FIF bioreactors. The improved bioreactor showed better Oxygen transfer rate from Compartment C to Compartment A&B and neither gas nor liquid leakage between the compartments. Improved FIF bioreactor has been chosen for future works. Future experiments will study relationship between the flow rate and the Oxygen Transfer Rate (OTR) and The hydraulic permeability values of the inner fibers. Also a mathematical model will be developed to predict oxygen transfer in the FIF bioartificial liver device. The model parameters will be taken from the constructed and tested FIF modules. Cellular oxygen uptake was based on Michaelis-Menten kinetics and commercially available Matlab software will be used to detect the mathematically the OTR behavior of the device and will be compared to the experimental results.

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