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Engineering Analysis and Development of the Spheroid Reservoir Bioartificial Liver

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***Abstract*—A significant demand exists for a liver support device such as a Bioartifical Liver (BAL) to treat patients experiencing acute liver failure. This descriptive paper outlines the design and development of two of the key components of the Mayo Spheroid Reservoir Bioartificial Liver (SRBAL) system. One of the components is the multifunctional Spheroid Reservoir and the other is Multi-shelf Rocker. The Spheroid Reservoir provides an environment to support the viability and functionality of the hepatocyte spheroids at very high cell densities. The Spheroid Reservoir is the biologically active component of this extracorporeal liver support device. Since the Spheroid Reservoir is designed to support 200-400 grams of hepatocyte spheroids, a method to quickly produce large quantities of spheroids is required. The Multi-Shelf Rocker fulfills the production requirement by allowing the culturing of up to six liters of hepatocyte suspension in a conventional laboratory incubator. The SRBAL is designed to provide life sustaining liver-like function to patients in acute liver failure.**

I. Introduction

LIVER failure is a serious problem with an annual incidence exceeding 200,000 cases and an annual mortality exceeding 40,000 deaths in the United States alone [1]. Other than liver transplantation, which is limited to donor organ availability, no adequate therapy exists for supportive treatment of most forms of acute liver failure. Liver transplantation is further complicated by the side effects of anti-rejection drugs required for graft protection. One solution to the limitations of liver transplantation is a cell based liver support device to treat patients with acute liver failure, either as a bridge to liver transplantation or until spontaneous recovery of the liver.

In the past, first-generation cell based artificial livers have been limited by their dose of hepatocytes and their duration of therapy. These devices used anchorage-dependent liver epithelial cells attached to materials such as synthetic polymeric membranes, nanofiber scaffolds [2] or hollow fiber filters [3]. Unfortunately, due to issues related to membrane pore size, mass transport, surface properties, and the restricted number of liver cells supported, these devices have shown limited success [4], [5]. Issues of insufficient hepatocyte mass and short duration of therapy have been the incentive to develop the Spheroid Reservoir Bioartificial Liver (SRBAL).

As reported by Nyberg, et al. [6], a means to rapidly create spherical aggregates of pig hepatocytes (i.e., spheroids) has been devised. The spheroids are cultured in suspension and do not need an attachment surface or scaffolding to remain viable. By maintaining hepatocyte spheroids in a high cell density suspension culture, large quantities of hepatocytes can be incorporated into a bioartificial liver without changing the blood volume of the extracorporeal circuit. These hepatocyte spheroids are functional live liver sub-units [7]. They have been shown to detoxify undesirable toxins through cytochrome P450 activity and provide metabolic functions such as protein synthesis, urea production from ammonia (ureagenesis), and conversion of lactic acid to glucose [5].

Previously reported methodologies for producing and culturing hepatocyte spheroids supported cell densities of 0.5×10^6 cells/ml [8]. It is estimated that to make an effective Bioartificial Liver (BAL), cell densities of 1-2 $\times10^7$ cells/ml are needed. This requirement brings to light two issues in creating a practical BAL; how to make enough spheroids and how to keep the spheroids viable in a high density suspension.

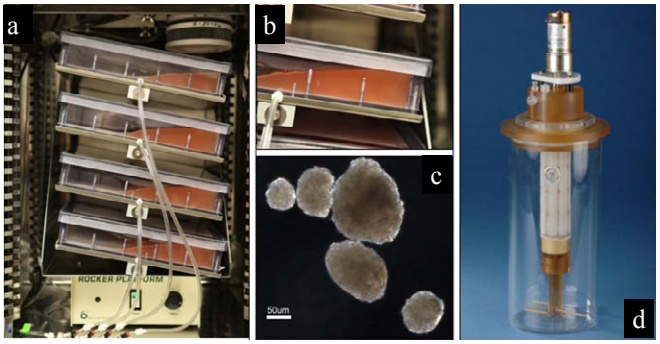

Fig. 1. Multi-shelf rocker (a and b), hepatocyte spheroids (c), and prototype impeller-agitated spheroid reservoir (d).

In order to culture a large quantity of hepatocyte spheroids, a newly developed Multi-Shelf Rocker was built

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(see Fig. 1a and 1b) that can produce up to six liters of hepatocyte suspension.

The requirement for a high cell density Spheroid Reservoir can be met by the design and development of an impeller-agitated spheroid reservoir.

II. Multi-shelf Rocker

Rocking bio-reactors have been used to rapidly induce hepatocyte spheroid formation [8]. The current design is the fourth generation rocking bio-reactor used to produce spheroids. This design has scaled the production capability of hepatocyte spheroids from 400 ml to 6000 ml. Fig. 2 and Fig. 3 illustrate the four tier, multi-shelf rocker system. The system was design to be integrated with a Bellco standard rocker (7740-10100) and placed inside an incubator at 37°C during spheroid production. Each of the four spheroid bio-reactors are supplied with a 95% oxygen, 5% carbon dioxide gas mixture. The current rocking speed has been set to 15 cycles/min with a 7° range of motion. The semi-permeable membrane used is silicone rubber (nominally 127 µm thick) reinforced with polyester mesh. This membrane allows gasses such as oxygen and carbon dioxide to pass into the cell media but is impermeable to fluids. Each spheroid generation chamber can support 1500 mL of hepatocyte suspension at 5×10^6 to 10×10^6 cells/ml.

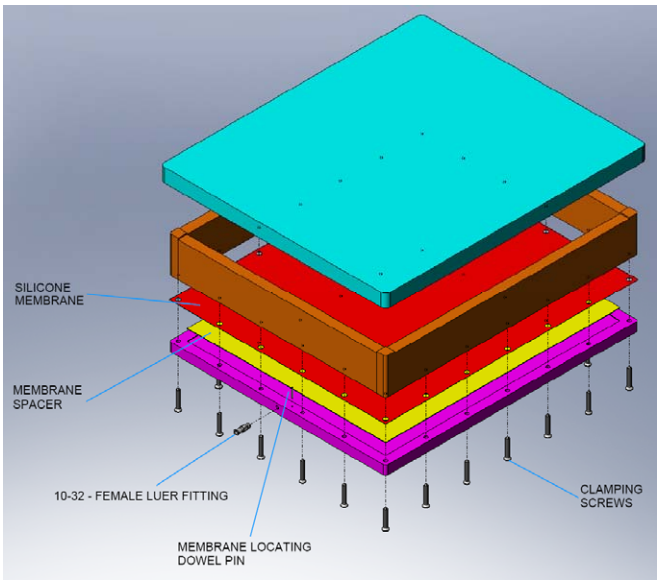

Fig. 2. Spheroid generation chamber assembly.

Testing has shown that the multi-shelf rocker system can produce spheroids with average diameters of 70 to 100 µm in 12 hours. Currently, the multi-shelf rocker is being used to produce porcine hepatocyte spheroids to support ongoing testing and design optimization of the impeller-agitated spheroid reservoir.

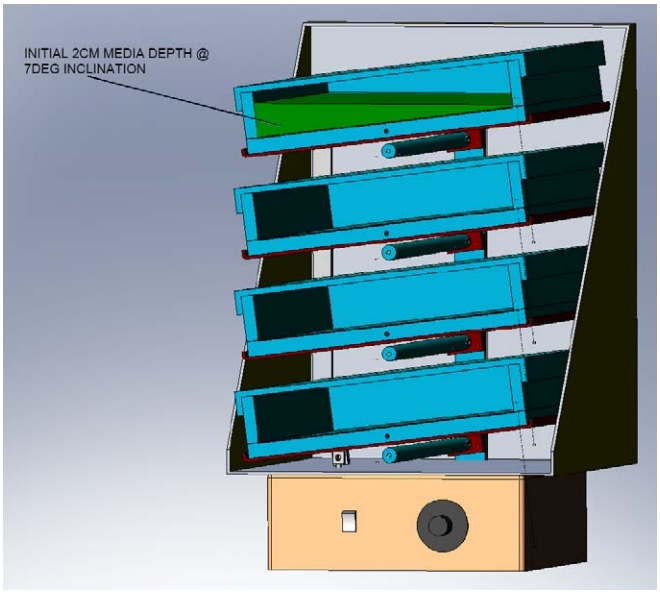

Fig. 3. Multi-shelf rocker assembly.

III. Prototype Impeller-Agitated Spheroid Resevoir

While a rocking bio-reactor is well suited for production of hepatocyte spheroids, an impeller-agitated type spheroid reservoir is better suited to be scaled up for clinical application of the SRBAL. This is due to the multi-shelf rocker being a reciprocating device that necessitates a large footprint to support the required numbers of hepatocyte spheroids. The impeller-agitated spheroid reservoir can be designed with a small footprint and still support high densities of hepatocyte spheroids. The impeller device can also be made to accept drop-in cell medium membranes that can easily be attached to a disposable tube set. Overall, the concept of the impeller design is more conducive to design for manufacturability and usability. Therefore, the development of a first generation full scale impeller-agitated spheroid reservoir has been undertaken.

Several prototype impeller-agitated spheroid reservoirs have been built. Prototypes, like the one shown in Fig. 1, have been tested and demonstrate good mixing using 10 to 50 and 100 to 500 µm polystyrene microspheres, each with a uniform size distribution. Prototypes were also tested with 70 to 100 µm diameter spheroids at 98 and 196 revolutions per minute (RPM). As shown in Fig. 4, this testing showed that at the lower RPM spheroids would aggregate together and at the higher RPM this was eliminated

Studies of Blood Urea Nitrogen (BUN) production indicate that the hepatocyte spheroids were alive and performing ureagenesis at both 98 and 196 RPM. Applying a linear fit to BUN concentration data taken at selected time intervals (time = 0, 4, 22 and 24 hours), BUN production rates of 7.2 mg/hr and 7.7 mg/hr were calculated for 98 RPM and 196 RPM respectively.

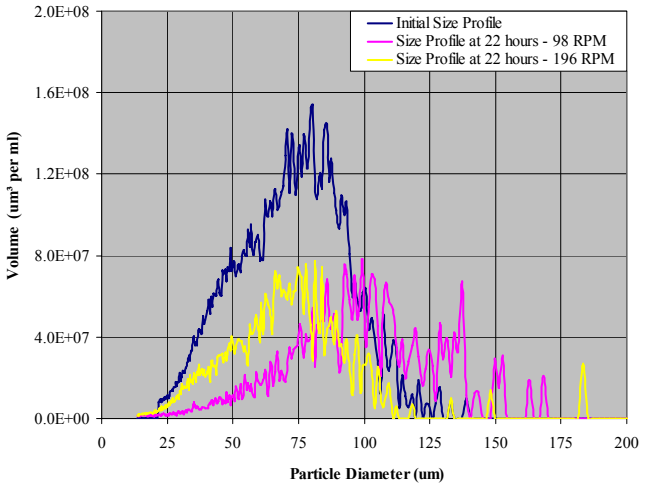

Fig. 4. Effect of impeller speed on spheroid size distribution.

The device was tested in an incubator to verify the oxygen transfer was sufficient to support large numbers of spheroids. The oxygen tension was monitored to determine if oxygen consumption was overcoming the amount supplied. This generation impeller-agitated spheroid reservoir can support 100 grams of cells per liter and requires a 200ml/min flow of oxygen gas. Carbon dioxide gas flow rates are continually adjusted via sensor feedback to maintain a constant pH of 7.40.

Additional testing with live porcine hepatocyte spheroids showed that further design optimization was required to prevent clogging of screen filters used to keep spheroids in the impeller-agitated spheroid reservoir.

The spheroid reservoir design increases therapy duration by supporting continuous perfusion as well as real-time monitoring of oxygen consumption and hepatocyte spheroid viability. The device also supports replacing a bioreactor showing signs of decreased oxygen consumption or reduced hepatocyte spheroid viability without terminating BAL treatment.

IV. Self Cleaning Spin Filter

The next generation design consists of a 3 liter cylindrical vessel with an inside diameter of 11.4 cm and a height of 35 cm. The vessel is lined with a nylon screen which acts as a gas distribution path. Inside the support screen is a semi-permeable membrane bag fabricated from 127 µm thick silicone rubber reinforced with polyester mesh. Cell media and previously generated hepatocyte spheroids are loaded into the semi-permeable membrane bag. The vessel has gas input and exit ports that allow mixtures of oxygen and carbon dioxide to flow over the membrane enabling the transfer of oxygen to the spheroid suspension and control of pH. The vessel's open top is sealed with a lid that is equipped with a temperature probe port and a reservoir sample port. Centered on the lid is a non-rotating cylinder, 18.4 cm long and 0.953 cm in diameter. This cylinder has two slots machined down its length. A drive shaft is connected to a motor mounted on the lid. This drive shaft turns a rotating outer cylinder that rides on the non-rotating cylinder and an impeller.

Fig. 5. Next generation spheroid reservoir with self cleaning spin filter.

As shown in Fig. 5, the outer cylinder has six rectangular windows machined into it. As the outer cylinder rotates around the central cylinder, in-flow and out-flow slots are opened by the rectangular windows. Cell media is circulated into and out of the reservoir via these openings. The in-flow slot cut into the central cylinder is 0.079 cm in width and 3.0 cm long. The larger out-flow slot is 0.635 cm in width and 3.0 cm long. A screen filter with 20 µm pores is mounted to a 0.038 cm thick Teflon® bearing surface that rides between the central cylinder and the windowed outer cylinder. As the filter screen rotates, it is continually being cleared of debris by the flow of fluid passing through the in-flow or the out-flow slots. The impeller is located at the bottom of the reservoir and rotates with the outer cylinder. If the system is operated between 100 and 200 RPM, the impeller provides sufficient agitation to ensure good mixing of the culture medium and keeps the spheroids in suspension.

Known limitations of the self cleaning spin filter include the engineering trade-off between Teflon® bearing thickness and volumetric shunting of cell media from the in-flow to the out-flow ports, the maximum attainable flow rate due to adhesion of hepatocyte spheroids to the filter screen, and the need to perform bench testing with hepatocyte spheroids due to the unavailability of representative particulates with nearly equivalent adhesion and elastic properties.

V. Conclusions

At this time, a multi-shelf rocker system has been developed and shown to produce large quantities of liver spheroids. This device in conjunction with an impeller-agitated spheroid reservoir will enable development of a cell based SRBAL. Remaining future work includes advancing the impeller-agitated spheroid reservoir design to optimize the filter screen size, eliminating filter clogging, and selecting the best flow rate. This spheroid reservoir will then be integrated into a complete SRBAL system that includes a modified commercially available dialysis machine to manage body fluids extracorporeally. A series of pre-clinical studies will be performed using a canine model of drug-induced acute liver failure to assess efficacy of the SRBAL.

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