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Short Communication Open Access

The Hollow Fiber Infection Model: Principles and Practice

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Short Communication

Emerging antibiotic resistance presents a serious global health threat. 2 million people in the United States were infected with antibiotic resistant bacteria in 2014 and more than 20,000 died as a direct result of these infections, many more from complications. Antimicrobial resistance has been identified as one of the three greatest threats to human health [1]. An urgent worldwide initiative is required to address the emergence of resistant strains of bacteria. The 10 × 20 Initiative aims to develop 10 new antibiotics by the year 2020 [2]. However, there are few new antibiotic candidates in the pipeline. There are at least three reasons for this: 1) Scientific: the easy-to-discover antibiotics have already been found; 2) Economic: antibiotics represent a poor return on investment and newly approved antibiotics are reserved for difficult cases, further reducing their profitability; 3) Regulatory: the FDA approval process is increasingly complex and expensive.

Antibiotic discovery and development require static susceptibility testing to screen compounds, *in vitro* pharmacodynamics/pharmacokinetic (PK/PD) studies to model drug dynamics and efficacy, and testing in animal models to provide critical information prior to the clinical evaluation of new antibiotics.

Animal models have many shortcomings though they have served as a primary development tool for many years:

- PK/PD may not match human values
- Cannot sample same animal over time
- Difficult to study large numbers of bacteria to reveal resistance
- Many infections cannot be modeled in a mouse or other animal

The one compartment PK/PD model typically consists of an open central reservoir containing the organism of interest, a source of diluent and a waste reservoir. Drug is added to the central reservoir as a bolus infusion. The drug elimination curve is modeled by adding drug free diluent to the central reservoir. Bacteria are also removed during this process. Current one compartment *in vitro* PK/PD models are not an effective method to properly mimic human PK/PD, particularly for drugs with a short half-life, requiring large amounts of drug and diluent. This open system results in bacterial loss that complicates data analysis. An open system also presents the risk of exposure to pathological agents.

- Open system, not bio safe
- Bacteria numbers change over time
- Large volume requires large amount of drug and diluent
- Rapid changes in drug concentration not possible, cannot model short half-lives

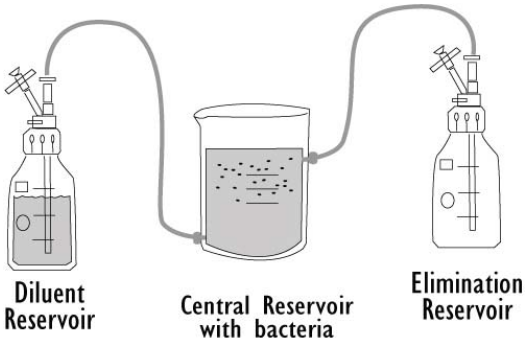

Figure 1: Diagram of the one compartment model.

The Hollow Fiber Infection Model

To address these shortcomings the two-compartment *in vitro* pharmacokinetic model utilizing hollow fiber bioreactors was developed, the Hollow fiber infection model (HFIM). Hollow fiber bioreactors are modules containing thousands of hollow fibers; small tubular filters 200 microns in diameter. The fibers are sealed at each end so that liquid entering the ends of the cartridge will necessarily go through the insides of the fibers. The pore size of the fibers is selected to retain the organisms while allowing drugs and other small molecule to freely cross the fiber. Bacteria or cells are inoculated on the outside of the fibers, trapped in the space called the extra-capillary space, or ECS. The ECS is defined by the space outside the fibers but within the cartridge housing. Medium from the central reservoir continuously recirculates through the inside of the fibers providing oxygenation and nutrition support. Small molecules such as drugs, glucose and metabolic waste products can easily cross the fiber while larger bacteria, cells and viruses cannot cross the fiber.

Hollow fiber cartridges were first used by Zinner [3] for bacterial testing in the 1980's and by Billelo et al., [4] for anti-HIV drugs in the 1990's. The HFIM model is in widespread use for both antibiotic testing [5] as well as anti-viral testing [6]. Hollow fiber cartridges offer the advantages of having an extremely high surface area to volume ratio, in excess of 150 cm^2 per milliliter of volume, providing rapid and uniform distribution of the drug within the ECS. Several different types of hollow fiber polymers are commercially available to allow for compatibility with drugs of different chemistries.

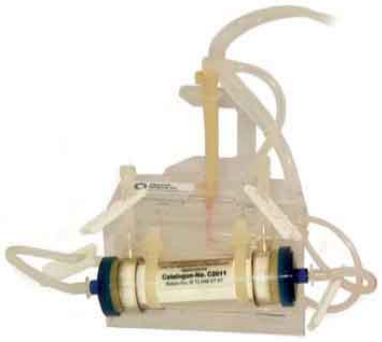

Figure 2: Hollow fiber cartridge as available from FiberCell Systems Inc. used in the hollow fiber infection model.

The design of the two-compartment model is quite similar to the one-compartment model except that the organism to be tested is confined within the small volume of the ECS (20 ml), physically separated from the central reservoir by the semi-permeable membrane. The concentration of the drug in the central reservoir equilibrates rapidly with the medium in the ECS containing the organisms, which is relatively small in volume. The volume of the central reservoir can be adjusted to permit rapid changes in drug concentration.

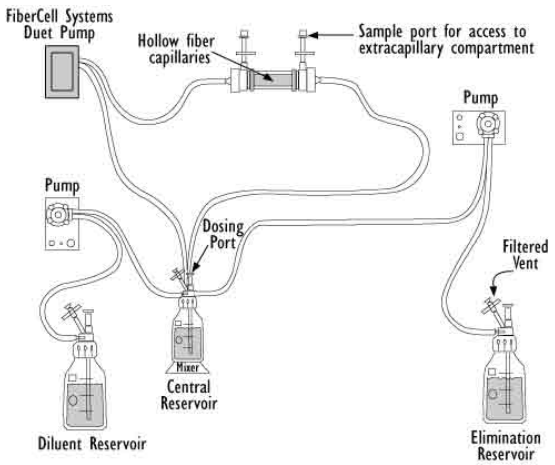

Figure 3: Diagrammatic representation of the hollow fiber infection model. Drug and diluent is added to the central reservoir to control the concentration of drug. Drug concentration in the central reservoir is equal to the concentration of drug the bacteria are exposed to in the extra capillary space of the hollow fiber cartridge.

Bacteria are retained in the small volume of the area outside of the fibers, the extracapillary space, while medium or broth containing varying amount of drug flow through the inside of the fibers. The drug rapidly equilibrates across the fiber.

The advantages of the two-compartment hollow fiber system are numerous. The target bacteria are contained within a very small volume, 10-20 ml, so they are at a similar concentration to *in vivo* infections and the drug can equilibrate rapidly within the compartment. Representative samples can be taken easily without significantly affecting the bacteria population. Drug resistant, highly pathogenic and highly bio hazardous organisms are safely contained in a sealed compartment. Large numbers of organisms can be tested in one experiment so the emergence of drug resistance is easily quantified. Both absorption and elimination kinetics of the drug being tested can be precisely and independently controlled and mimic human bioavailability. The kinetics of multiple drugs can also be controlled so drug/drug interactions and combination therapies can readily be examined. The system is compact enough that multiple cartridges can be conveniently manipulated in a relatively small space. The advantages of the HFIM are as follows:

- Closed, bio-safe system
- Large number of organism can be tested, revealing resistance
- Precisely simulates human PK/PD
- Repetitive sampling over time, both drug and organism
- Total kill can be determined
- Single use, disposable, reproducible
- Two drug models can be tested
- Can model both dosing curve and elimination curve
- Can look at bacteria in different growth phases and in combination with cells
- Antiviral PK/PD can be performed as well

Figure 4: One example of a hollow fiber infection model set up. Diluent bottles upper right, waste reservoirs below.

Setting up a System

Overview

The HFIM may appear to be complicated to set up but in fact the layout is quite simple. The central reservoir is fitted with a cap that has 5 tubes on it. Two of the tubes are connected to the hollow fiber cartridge and broth or medium from the central reservoir is recirculated through the cartridge and back into the central reservoir. Of the remaining tubes one is connected to the diluent reservoir for diluent addition, one is connected to the waste container and the final tube is fitted with a sterile filter and serves as a vent to prevent pressure

from building up in the central reservoir. This port can also serve as a dosing port. If an autoclave bottle for the central reservoir is used the entire tubing system, including the diluent and waste bottles and caps can be autoclaved as a single piece. The only connections that need to be made in the laminar flow hood are to the inlet and outlet from the hollow fiber module itself, using the luer connectors provided. The Fiber Cell hollow fibers modules are supplied pre-sterilized with all required tubing and are specifically designed to fit into the P3202 Duet pump. The duet pump will provide 80-120 ml per minute flow rate though the recirculation loops of the cartridge. A high flow rate is required to ensure rapid and uniform equilibration of the drug through the fibers of the cartridge and uniform distribution of the drug throughout the loop.

Figure 5: Another example of a hollow fiber infection model laboratory set up.

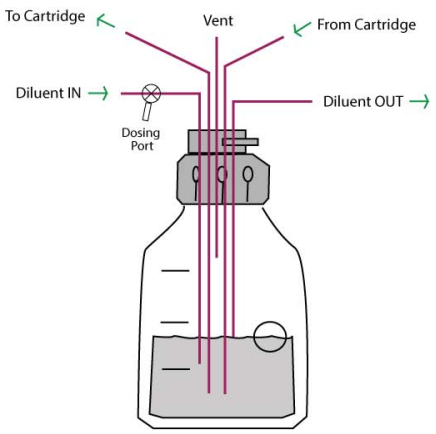

Figure 6: Schematic of the 5-port reservoir cap. The tubes can be moved up and down to set the level of the central reservoir.

Central reservoir

Drug is added to the central reservoir either as a bolus or with a syringe pump to model the absorption curve. Diluent is added to the central reservoir to reduce the concentration of the drug and mimic the elimination curve. The drug concentration is calculated to precisely model human absorption and elimination curves. The HFIM is based upon the fact that the concentration of the drug in the central reservoir is equal to the concentration of the drug in the extra capillary space of the cartridge, where the bacteria of interest are retained. Changes in the concentration of drug in the central reservoir equals changes in the drug concentration in the ECS, as the drug will equilibrate rapidly across the fibers. It is important for the volume of the central reservoir to be relatively small to allow for rapid changes in drug concentration and also to reduce the amount of drug and diluent required. A typical volume will be between 50 ml and 125 ml. It is preferable to use a bottle with a 38 mm neck size to accommodate the *in vitro* toxicology cap that is provided by Fiber Cell Systems Inc. Although other caps can be used, they should provide 5 ports with luer connections and adjustable tube length. Another important consideration for the central reservoir is that the volume must remain constant to keep the concentration of drug at a defined level. It is quite simple to ensure that this volume remains constant. Each cartridge will require two heads on a peristaltic pump, one head to add diluent and one head to remove diluent from the central reservoir. When setting up the system utilize the next size larger pump head tubing for the diluent removal head of the peristaltic pump. This will ensure that the rate of diluent removal is always slightly faster than the rate of diluent addition. Adjust the height of the "diluent out" tube so that it is at the specific level required for the broth in the central reservoir. The central reservoir volume will now remain constant at that level without further adjustment. The central reservoir bottle itself can be a pre-sterilized plastic bottle such as from Nalgene or an autoclavable plastic bottle such as from TriForest Enterprises (Irvine, Ca.). The autoclavable bottle can be inserted into the assembled system prior to sterilization to reduce risk of contamination. Sometimes a weighted ring is placed on the central reservoir bottle to prevent it from tipping over.

Figure 7: Example of the central reservoir with weighted rings to prevent the bottle from tipping over.

Dead volume

To calculate the concentration of the drug it is important to take into account the dead volume of the system. For the C2011 20kld MWCO polysulfone cartridge the ECS volume is 20 ml, the volume of the ICS (intracapillary space) is 20 ml and the volume contained in the loop of tubing is 25 ml for a total volume of 65 ml in addition to the broth in the central reservoir. The ECS of the C3008 cellulosic cartridge is slightly smaller, 12 ml so the total volume is 49 ml.

Cartridge selection

The polysulfone cartridge, C2011 has superior flux characteristics and more uniform fiber geometry than the C3008 cellulosic cartridge and should be considered as the first choice when selecting a cartridge. However, if the drug being tested is strongly polar or requires a solvent such as DMSO or chloroform to get it into solution it may demonstrate non-specific binding to the polysulfone fibers, although this is not always the case. Large amounts of solvents may also have a negative effect on the integrity of the fiber structure so they should also be avoided with the polysulfone fiber. The cellulosic fiber is a good choice for these types of drugs.

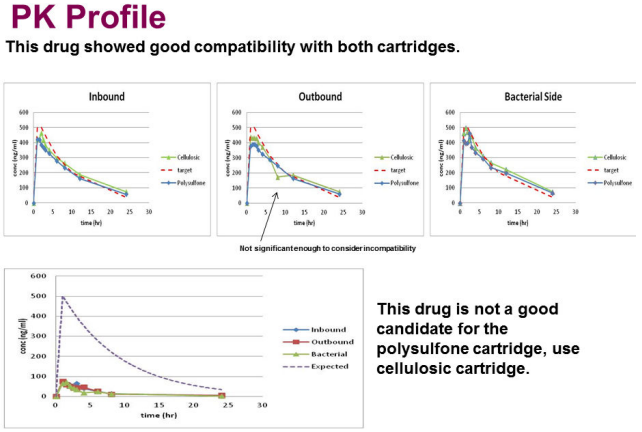

Figure 8: Demonstration of data showing good drug characteristics (no non-specific binding) and poor drug characteristics (non-specific binding of drug).

The polysulfone fiber is co-extruded with PVP (polyvinylpyrrolidone), an FDA approved blood expansion product, much like glycerin. The cellulosic fibers are treated with glycerin prior to irradiation. In both cases the cartridges should be pre-cultured overnight with broth to remove these agents. Prior to setting up the pre-culture the ECS of the cartridge should also be filled with broth, in the laminar flow hood using syringes.

Sampling

One of the prime advantages of the hollow fiber infection model is the ability to take multiple samples over many time points, and also to be able to run the study for several weeks if desired. Since there are antibiotics in the system it is not necessary to pull the system out of the incubator to perform sampling. Samples can be taken with a 5 ml, 10 ml, or 20 ml syringe, one attached to each side port. There are many different ways to sample but the simplest way is to attach a syringe to each side port and gently pull one ml into one of the syringes. Close the end ports of the cartridge and flush the sample back and forth 3-4 times in order to mix the contents of the ECS. Push the entire sample into one syringe and remove it. Replace with a fresh syringe. Replacing the luer caps on the side ports with Clave fittings can provide an extra bit of protection. These will allow sterile samples to be taken easily and reduce any risk of contamination of the cartridge.

Figure 9: The clave fitting, available from Hospira Inc.

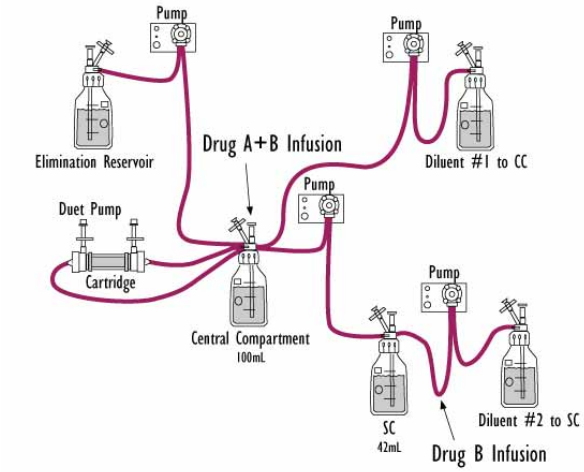

Figure 10: Diagram of the two drug model. The drug with the longer half-life is added in a controlled manner to the diluent.

Two Drug Model

Another strength of the hollow fiber infection model is its' ability to easily model combination therapies and two drug models, as shown in the diagram below. In this case the drug with the longer half-life is added to the diluent while the drug with the shorter half-life is eliminated. The precise definition of two drug combination therapies can play a critical role in defining therapeutic regimens to combat resistance strains of bacteria.

Incubator

Bacterial broths generally do not use a carbonate buffering system so a CO_2 incubator is not required. A large standard incubator can be used to support up to 8 duet pumps and 16 cartridges at one time. The

diluent reservoirs can be maintained outside of the incubator, as flow rate of the diluent should not be high enough to affect the temperature of the central reservoir. Small slits can be cut in the gasket of the door to allow tubing to enter and exit the incubator. An anaerobic chamber can also be used as an incubator as shown below.

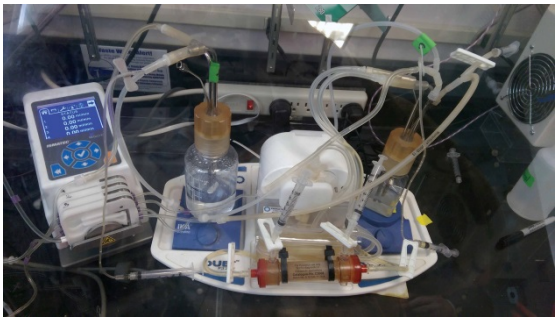

Figure 11: The hollow fiber infection model can be set up in an anaerobic chamber to control gas composition.

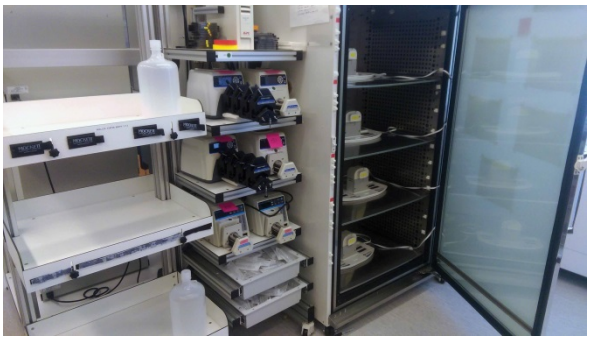

Figure 12: An example of another HFIM model set up. There are three wheeled carts, one for the incubator, one for the peristaltic pumps and one for the diluent and waste.

There are three wheeled carts, one for the incubator, one for the peristaltic pumps and one for the diluent and waste making it easy to move them around the laboratory. In this case the drug is light sensitive so the glass door of the incubator is covered.

Summary

The hollow fiber infection model is not intended to be a replacement for static susceptibility testing, animal testing nor clinical trials. However, there are many advantages vs. animal models and current *in vitro* models. Recently the European Medicines Agency actively endorsed the hollow fiber infection model as a validated method for generating data to support drug submissions. The FDA is expected to follow suit. The growing reference library on the hollow fiber infection model and continuing demonstration of its validity with reference to clinical outcomes highlights its value both in pre-clinical studies and in the post-approval setting, especially for revealing the development of resistance, optimal dose selection and route of administration

- Optimal dosing schedule
- Possible combination therapies
- Define dosage profiles that result in resistance
- Post-approval drug regimen optimization
- Can support trial design for Phase I, II, III and IV clinical trials

The hollow fiber infection model is a complementary and additional tool for antibiotic development. In the current antibiotic "age of resistance" it serves as an important tool in the development of new antibiotics as well as new applications for current drugs.

Acknowledgment

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