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Faster Development of Anti-Infective Therapies

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THE HOLLOW FIBRE INFECTION MODEL

Antimicrobial resistance (AMR) is one of the biggest health threats worldwide and necessitates the urgent development of novel, broad-spectrum antibiotics that are able to address the challenges of AMR and counter some of today's most serious microbial threats. Key to de-risking and expediting the development and approval of new antimicrobials is a detailed understanding of the relationship between the fate of the antimicrobial compound in the body (pharmacokinetics; PK), and the impact of exposure to the compound on the target microbe (pharmacodynamics; PD). Such an understanding informs the development of optimal human dosing regimens that maximise efficacy and minimise the emergence of resistance, thereby mitigating the risk of clinical trial failure, and ultimately extending the clinical utility of the new antimicrobial in the face of increasing AMR. Several non-clinical *in vivo* and *in vitro* models of infection exist that provide complementary information towards understanding this PK/PD relationship, amongst the most versatile of which is the *in vitro* hollow fibre infection model (HFIM), which will be described in detail in this paper.

THE IMPORTANCE OF NONCLINICAL PK/PD

The ultimate goal of nonclinical PK/PD in the anti-infectives area is to understand antimicrobial exposure – microbial response relationships, which then enable us to optimise dosing regimens to maximise the efficacy of an antimicrobial compound (microbial killing), minimise toxicity and - importantly in the era of AMR - minimise the risks of the emergence of resistance to the antimicrobial.

There has been an increasing emphasis on nonclinical PK/PD in recent years, driven by several factors including

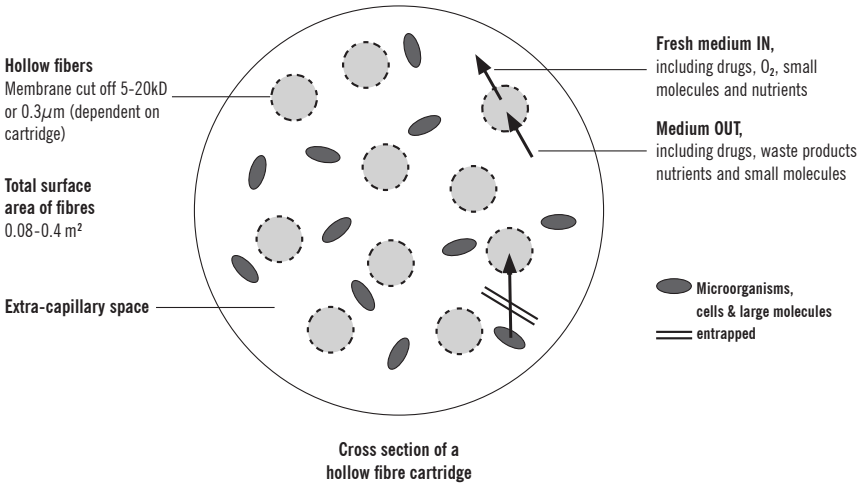
- guidance from regulatory authorities including the European Medicines Agency (EMA)[1] and the Food and Drug Administration (FDA) in the United States;
- abbreviated development paths for antimicrobials to address unmet clinical needs;
- lack of clinical trial feasibility or limited availability of clinical data due to the difficulty of recruiting sufficient numbers of patients with specific types of infection, e.g. multi-drug resistant bacteria, or patients infected with specific organisms in the case of narrow-spectrum antimicrobials;
- label expansion for an antimicrobial already in clinical use;
- adjustment of dosing regimens, for example to suit special patient populations or to suppress the emergence of resistance and extend the clinical utility of an antimicrobial;

The Hollow Fibre Infection Model (HFIM) provides a sophisticated solution to address these requirements.

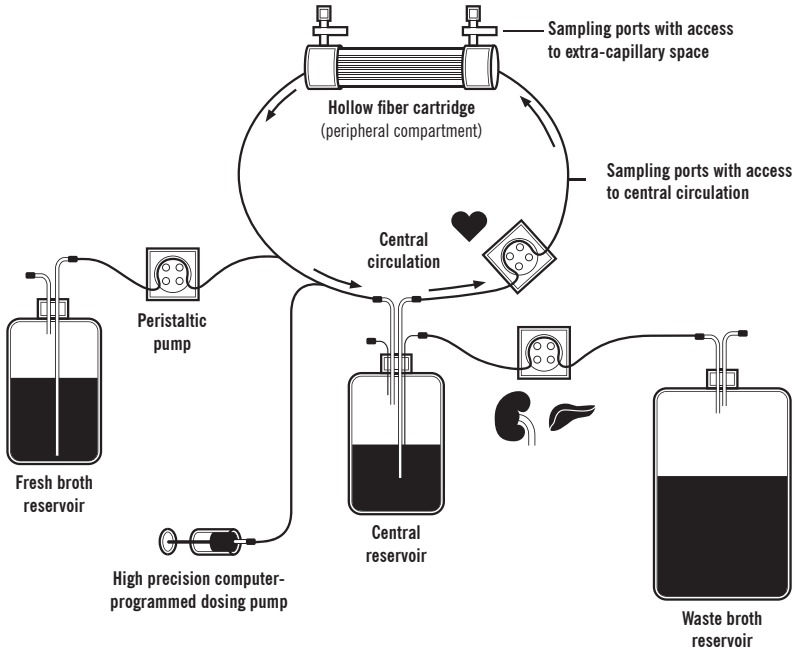
WHAT IS THE HOLLOW FIBRE INFECTION MODEL?

The HFIM is an *in vitro* system consisting of two principal compartments:
(i) a central reservoir and associated tubing, which constitutes a circulating system, and
(ii) a hollow fibre cartridge.

The cartridge consists of a sealed tube housing thousands of hollow permeable capillaries, or fibres, with defined molecular weight cut-offs, that allow for physical separation of the central compartment from the extra-capillary space (ECS) in the cartridge. The latter represents a peripheral infection site containing the target microorganism. Computer-controlled infusion pumps are used to precisely administer antimicrobial compound(s) into a fixed volume of growth medium in the central reservoir. The medium in the central reservoir is pumped to the hollow fibre cartridge, or eliminated to a waste reservoir, and is continually refreshed from an external supply. On entering the cartridge, growth medium containing the antimicrobial compound passes through the fibres and is able to diffuse into the ECS, ensuring continuous refreshment of nutrients and oxygen, removal of waste, as well as equilibration of the antimicrobial. Because of the molecular weight cut-off of the fibres, the bacteria are trapped in the ECS and unable to pass back into the circulating medium.



Cross section of a hollow fibre cartridge *showing fibres for medium in and outflux as well as microorganisms/cells and large molecules trapped in extra-capillary space*



The Hollow Fiber Model and its compartments. *Test cells / microorganisms are retained in the hollow fiber cartridge. The nutrient broth from the central reservoir is continuously in circulation. Waste can be removed and replenished by fresh broth so that the volume of the central reservoir is kept constant. Ports in the central circulation allow for sampling and the addition of test substances.*

By balancing the rate of infusion into, and the rate of elimination from the central circulation, the concentration of antimicrobial to which the microbes in the ECS of the hollow fibre cartridge are exposed can be precisely controlled. This dynamic system enables the HFIM to mimic almost any PK profile, including the PK of antimicrobial compounds observed in human patients.

Repetitive sampling for bioanalysis of antimicrobial concentration and bacterial load are made possible through sampling ports located on the cartridge, allowing the concentration of the antimicrobial in the cartridge (PK) as well as the impact of the antimicrobial on the target microbe (PD) to be monitored.

WHY CHOOSE THE HFIM?

The HFIM is the most capable *in vitro* system for the determination of *in vitro* PK/PD relationships between antimicrobial compounds and bacteria, fungi or viruses. It is a dynamic model that is able to simulate almost any given concentration-time profile for an antimicrobial compound or combination of compounds, without the constraints of animal PK, even if the compounds have very different half-lives. The bacteria are confined within the ECS of the hollow fibre cartridge so there is no risk of dilution of the culture. Indeed, the large surface area to volume ratio afforded by the fibres allows very high culture densities to be reached, often higher than those that are achievable with *in vivo* infection models, which is vital for resistance suppression studies that might predict clinical failure[2]. On completion of an experiment the cartridge can be drained, which makes it possible to demonstrate complete eradication of a microbial population. Another major advantage of the HFIM over *in vivo* models of infection is that the choice of microorganism is not limited by the availability of a validated animal model. Multiple cartridge types with fibres manufactured from different materials are available for use, so it is possible to optimise the HFIM for microbial growth and compound performance.

While *in vitro* assays such as the static concentration time-kill assay can provide early information on the time- or concentration-dependent bactericidal activity of a compound, their utility is limited because they are unable to mimic changes in compound concentration over time, as would naturally occur in a patient. One compartment models (those with just a central reservoir), such as a chemostat, allow for dilution of the compound to cater for a fixed half-life, but this unavoidably results in dilution of the bacterial culture, a problem that is exacerbated for compounds with short half-lives, resulting in loss of potentially resistant sub-populations.

Studies with the HFIM can be performed over much longer time periods than are possible with simpler systems as described above. This is extremely important for understanding PK/PD relationships and the risks of the emergence of resistance in response to a given treatment regimen over clinically relevant treatment time frames.

The HFIM is particularly useful for the development of antibiotic combination therapies. Selected examples include

- the combination of levofloxacin and imipenem prevented the emergence of drug resistance from clinical isolates of *Pseudomonas aeruginosa* even when subpopulations resistant to both drugs were present[3];
- relative to monotherapies, combinations of ampicillin, fosfomycin and ciprofloxacin delayed the emergence of antibiotic resistance development in *Escherichia coli*[4];
- different targets for the area under the concentration-time curve over 24h/MIC ratio (AUC_{24}/MIC) were required for different bacteria to develop quinolone-resistant subpopulations when exposed to garenoxacin[5].

Due to the ability to support the growth of eukaryotic cells to high densities in continuous culture, the Hollow Fibre System also lends itself to the study of antiviral compounds[6]; the sealed nature of the hollow fibre cartridge also affords an additional layer of biological protection. Studies have been published with multiple viruses including HIV, vaccinia, influenza, Zika, Dengue and Chikungunya.

The ability to run long-duration experiments with multiple drug infusion profiles means that the HFIM is especially well-suited to the development of antimicrobial combination therapy against slowly replicating bacteria such as *Mycobacterium tuberculosis*. During infection in patients, *M. tuberculosis* can exist in three metabolic states (dormant, semi-dormant, replicative) and in both extra- and intra-cellular compartments; the HFIM facilitates replication of these conditions *in vitro*. The use of the HFIM in anti-mycobacterial drug development is supported by the European Medicines Agency (EMA), who in 2015[7] published a Qualification Opinion stating the Hollow Fibre System of TB (HFS-TB) was *"qualified to be used in anti-TB drug development programs as an additional and complementary tool to existing methodology to inform selection of dose and treatment regimen, including combination of 2 or more anti-Mtb drugs, to maximize bactericidal effects and minimize emergence of drug resistance. HFS-TB can be used in regulatory submissions throughout the drug development process for a product, especially for more informed design and interpretation of Phase I, Phase II and Phase III clinical studies."* The FDA also described its utility as part of the agency's updated guidance on TB drug development.[8]

The EMA stated that the forecasting accuracy rate of the HFS-TB was 94.4%, commenting that the "model is a drug development tool that is highly accurate for forecasting optimal drug exposures, drug doses, dosing schedules and appropriate drug combinations for anti-TB drugs/drug regimens".

WHEN SHOULD THE HFIM BE USED IN THE DRUG DISCOVERY PROCESS, AND WHAT FOR?

The HFIM allows for exquisite control of kinetic parameters and the study of their consequent impact on pharmacodynamic efficacy. It can be positioned in different stages in the drug discovery process and provide different pieces of information of the potential use of the antimicrobial. This will be largely dependent on the information for the candidate compound that is available at each stage, in particular the available PK profiles in animals and humans, as this is required to model in HFIM.

- Early screening of compounds can de-risk future HFIM experiments. Some drug properties complicate handling in the system e.g. lack of solubility in biological medium at required concentration, or high levels of binding to system components. Equally, not all organisms are able to grow in the HF system. Finding out early that the HFS is not an option shifts the focus to other models for PK/PD assessment. At this stage the PK profile characteristics of individual drugs can be established and early resistance studies can be performed.

- Studies at the pre-clinical stage (pre-IND) will help to improve understanding of the PK/PD relationship. This is particularly important if an animal model is not available, or if the animal can't tolerate the inoculum levels that need to be tested (e.g. resistance studies). At this stage animal PK profiles or human concentration-time profiles derived from allometric scaling can be established. In addition, compounds can be assessed as monotherapies followed by combination therapy to investigate the contributions of individual compounds. Dose response studies can help minimise dose finding studies in clinical trials and dose fractionation studies will help to determine PK drivers of efficacy. Finally, resistance generation / mutant prevention window studies can be performed by modelling multiple dose regimens to assess the risk of selection of resistance.

- During clinical development the HFIM can help to inform trial design or build on what is already known. Hollow fibre studies can be established based on human data to address any major discrepancies between clinical and pre-clinical findings. Data generated in HFIM can particularly help where it is difficult to recruit sufficient patients to perform a clinical trial (e.g. for narrow spectrum antibiotics or MDR infections). Finally, information from HFIM studies can be used to identify or confirm optimal doses and combinations for Phase II and III studies

- Even after approval when the drug is in clinical use (phase IV) the HFIM can be used to expand the label, and/or to optimise the dosing regimen by generating profiles for special populations e.g. paediatric medicine and critically ill patients.

EVOTEC'S HFIM CAPABILITIES

Evotec has developed its own substantial HFIM offering in dedicated, state-of-the-art Containment Level 2 facilities at Alderley Park in the UK. With a growing team of scientists trained to operate the system and provide full microbiological support to HFIM projects, and supported by specialist bioanalytical and mathematical modelling and simulation teams, Evotec can offer its customers a bespoke *in vitro* PK/PD service tailored to advance their individual antimicrobial development programs.

In concert with its unique EvostrAIn™ collection of more than 8,000 reference and clinical isolates of microorganisms, Evotec is able to develop new HFIMs using strains with a wide range of antimicrobial susceptibility profiles and well-characterised resistance mechanisms that a novel antimicrobial may be expected to encounter in the clinic. The collection includes strains that have been validated for use in *in vivo* models of infection, ensuring continuity from first susceptibility profiling through to later stages of development.

Evotec have already performed HFIM studies, ranging in duration from a few hours to six weeks, using a range of microorganisms including

- Gram negative bacterial pathogens such as *Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli* and *Klebsiella pneumoniae*;
- extracellular *M. tuberculosis* (H37Ra, avirulent strain) in both the replicative and semi-dormant metabolic states;
- intracellular assays using THP-1 monocytes are currently in development;
- fungal pathogens, e.g. *Aspergillus fumigatus*.

Evotec´s growing experience includes the use of monotherapies, β-lactam / β-lactamase inhibitor combinations, and in the case of *M. tuberculosis*, combinations of up to 4 antimicrobial agents including the standard of care combination HRZE (isoniazid, rifampin, pyrazinamide, ethambutol). The team is experienced with handling demanding antimicrobial compounds that require optimisation of the Hollow Fibre System prior to use to ensure compatibility.

A variety of PD outputs from the HFIM are available or can be developed for use including

- enumeration of bacteria (total viable count) including dual-plating onto drug-free and drug-containing agar to monitor potential emergence of resistance;
- metabolic endpoints as an alternative to total viable count;
- detailed characterisation of resistant mutants;
- whole-genome sequencing of resistant mutants.

A typical workflow for a new HFIM project is outlined below, which would be tailored to meet the requirements of the customer and is responsive to the data generated at each stage:

1. Preparation phase

a. *In vitro* susceptibility testing, selection of appropriate strains and profiling of compound(s), spontaneous frequency of resistance experiments (depending on study objectives)
b. Compound solubility and stability under test conditions
c. Transfer or establishment of LC-MS bioanalytical assay

2. Hollow Fibre System set-up

a. Selection of hollow fibre cartridge type and compound compatibility / recovery experiments
b. Simulation of target PK profile and target attainment in the Hollow Fibre System

3. Pilot studies

a. Microbial growth in the HFIM
b. Dose-response studies to assess compound efficacy in the HFIM
c. Emergence of resistance studies, if required

4. Evaluation studies

a. Dose-fractionation studies to evaluate the potential pharmacodynamic drivers of antimicrobial activity

5. Resistance suppression

a. Evaluation of the impact of specific dosing regimens on the emergence of resistance
b. Determination of the magnitude of drug exposure required to suppress the amplification of resistant subpopulations of the target microbe

SUMMARY

Evotec's comprehensive HFIM capabilities combined with its drug development expertise and unique EvostrAIn™ collection of microbial pathogens provide a versatile *in vitro* PK/PD platform to de-risk and accelerate the development of antibacterial, antifungal and antiviral compounds. This makes Evotec´s HFIM offering an ideal state-of-the-art solution to contribute to the fight against antimicrobial resistance.

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