

Background

Fungal meningitis (FM) is an infection of the meninges of the central nervous system that manifests from the dissemination of any major fungal pathogen into the subarachnoid space (SAS) via the cerebrospinal fluid (CSF). Cryptococcal Meningitis (CM) is caused by *Cryptococcus neoformans* and is the most common cause of fungal meningitis in adults. Treatment for CM is based on an induction, consolidation, and maintenance approach with antifungals and is well-defined elsewhere^{1,2}, but is associated with continued high morbidity and mortality. Drug discovery programs are limited by poor penetration of the Blood Brain Barrier (BBB). Because of this, we developed an alternative catheter-based extracorporeal filtration system (Neurapheresis™ Therapy) for the filtration of infected CSF. **Here we describe the *in vitro* characterization of Neurapheresis™ Therapy as an alternative mechanical intervention for filtration of *C. neoformans* cells, polysaccharide antigen, and inflammatory mediators from infected CSF.**

Methods

In Vitro Growth of *C. neoformans*

H99, a clinical strain of *C. neoformans*, was grown overnight in 5mL YPD (yeast peptone dextrose) at 30°C for cell proliferation. Cells were transferred to 25mL of diluted Sabouraud/MOPS media and incubated for 24 hours at 37 °C to induce capsule growth. Cells were diluted to clinically relevant concentrations (1x10⁷ and 1x10⁶ cells/mL) for experiments.

Benchtop Filtration

Cells were diluted to clinically relevant concentrations in 150 mL of Sabouraud/MOPS and passed through the closed-loop Neurapheresis system equipped with either 100kDa or 5kDa tangential flow filters (TFF). The setup is demonstrated in **Figure 1** and consists of an "inlet" line leading from the sample reservoir to the filtration unit, the filter assembly itself, a device data recorder, a waste line for concentrated removal of organism, and an "outlet" line with a split allowing both clean sampling of the "permeate" and feed back into the sample reservoir. Samples were taken every full CSF volume filtration cycle (150 mL) for quantification of yeast load, antigen, and cytokines. Infected human CSF was used to obtain cytokine data.

Assays

- Colony Forming Units (CFUs) were quantified to determine organismal concentration.
- Cryptococcal Antigen was quantified using a Lateral Flow Assay (LFA by IMMY)
- Cytokines were quantified by a Luminex assay.

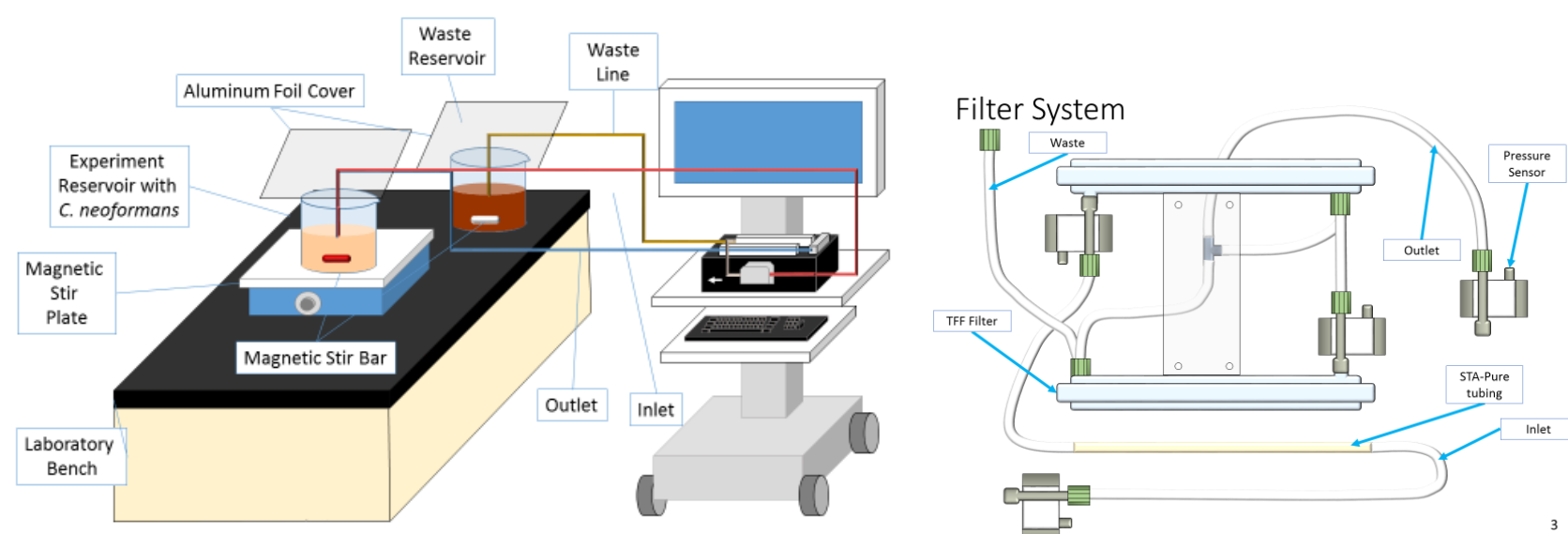


Figure 1. Schematic of the benchtop filtration experimental setup (left). Detailed schematic of the Neurapheresis filter assembly (right).

Results

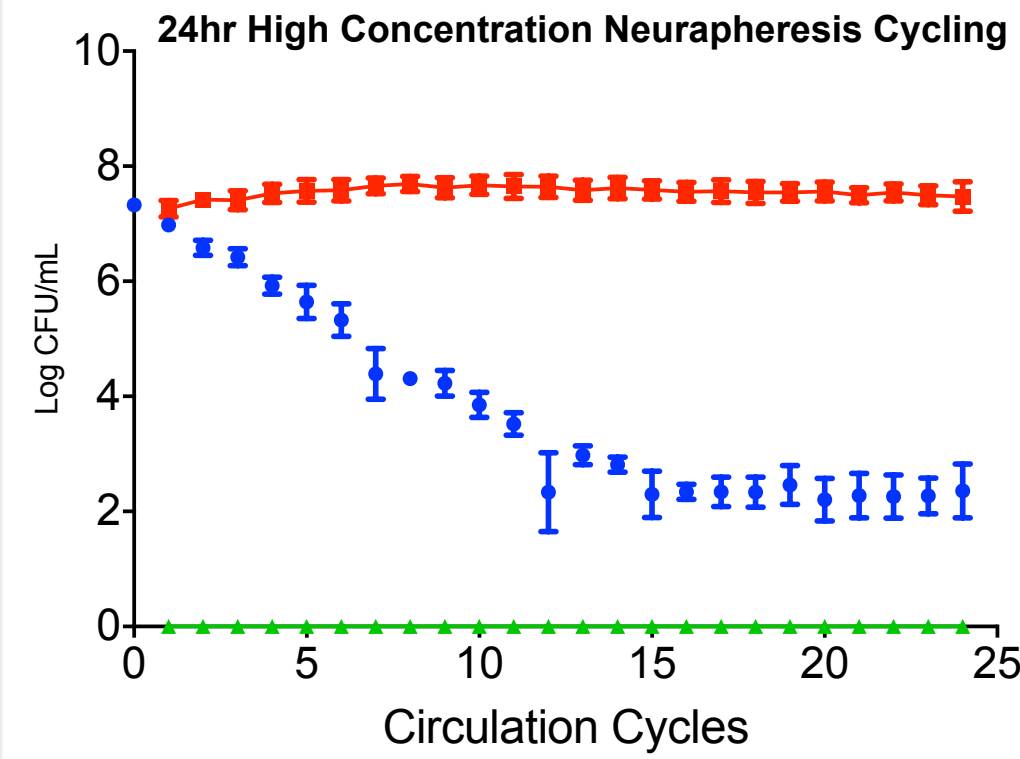


Figure 2. CFU reduction over 24 cycles of filtration through a 100 kDa TFF filter at a clinically high (1E7 cells/mL) organism concentration. A 5-log reduction was achieved over 24 cycles.

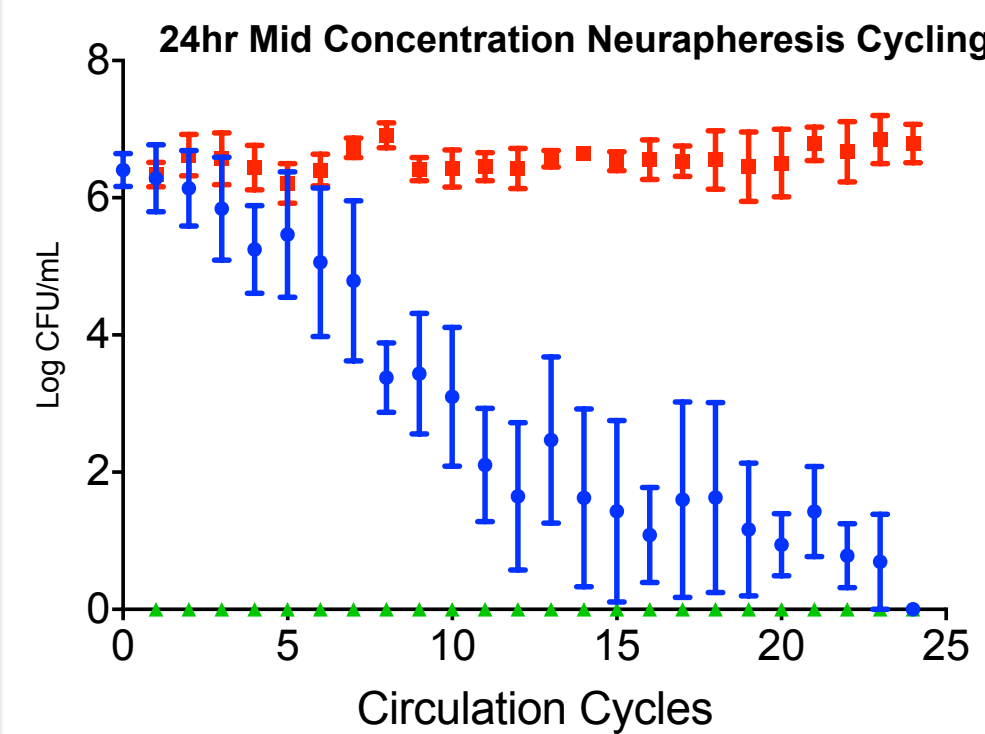
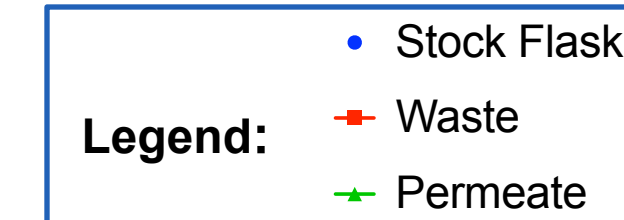


Figure 3. CFU reduction over 24 cycles of filtration through a 100 kDa TFF filter at a lower (~1x10⁶ cells/mL) organism concentration. CFU counts were reduced below the limit of detection in under 24 hours.

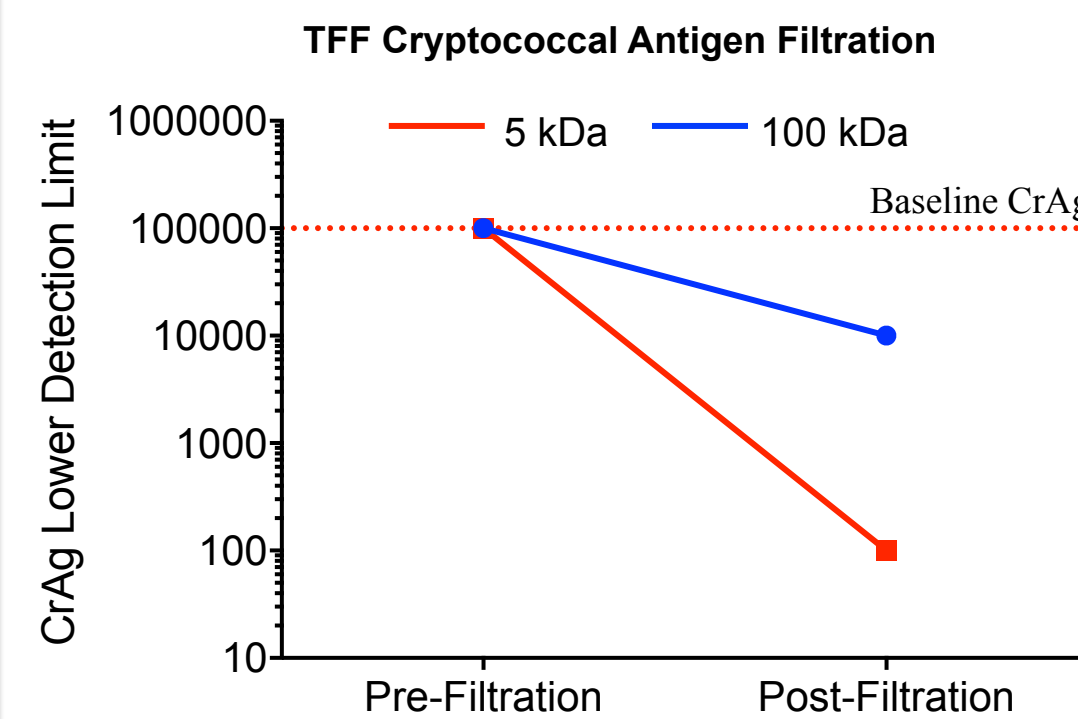


Figure 4. Cryptococcal Antigen (CrAg) was quantified before and after passage through 100 kDa (blue) and 5kDa (red) filter units. Samples were serially diluted and tested for the lower limit of detection with a CrAg LFA assay. Both 100kDa and 5kDa achieved antigen reduction ([initial titer]-[final titer]; [1:100000]-[1:10000] and [1:100000]-[1:100], respectively).

Cytokine/Chemokine Neurapheresis Filtration

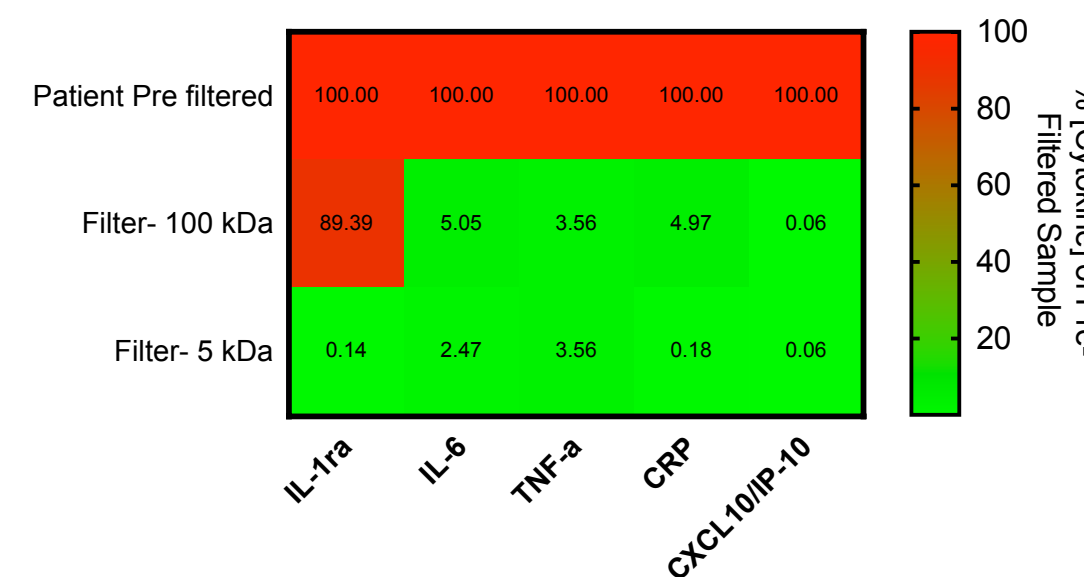


Figure 5. Infected human CSF was filtered using the Neurapheresis system and relevant cytokines were quantified. A reduction in cytokine levels (IL-1ra, IL-6, TNF, CRP, and CXCL10) was achieved. 100kDa reduced all cytokines except IL-1ra by >95% baseline, and 5kDa removed >95% of all cytokines quantified.

Conclusions

- Both tangential flow filter sizes cleared yeasts rapidly and efficiently.
- Over 24 cycles, we consistently observed a 5-log drop in CFUs (≥99%), reducing CFU counts below the limit of detection.
- 100kDa and 5kDa achieved a substantial antigen reduction (using CrAg LFA [initial titer]-[final titer]; [1:100000]-[1:10000] and [1:100000]-[1:100], respectively).
- A similar reduction in cytokine levels (IL-1ra, IL-6, TNF, CRP, and CXCL10) in infected human CSF was also achieved (100kDa reduced all cytokines except IL-1ra by >95% baseline, and 5kDa removed >95% of all cytokines quantified).

Neurapheresis™ Therapy is capable of substantially reducing CSF CFU burden in CM. Continuous filtration for multiple cycles shows promise for rapid CSF 'cleansing', and future iterations may include adjunctive infusions with drug therapies to rapidly and completely eliminate yeasts. **Substantial and rapid reduction of cryptococcal antigen and key inflammatory cytokines also has significant potential for controlling the neuro-inflammatory storm that accompanies CM.**

Future Directions

- Minnetronix is working to translate this research to humans. We are in development of a Neurapheresis system tailored to CM (**Figure 6**) for future clinical evaluations.
- A first-in-human clinical trial is currently underway to evaluate the safety of the catheter-based extracorporeal system in a different patient population.

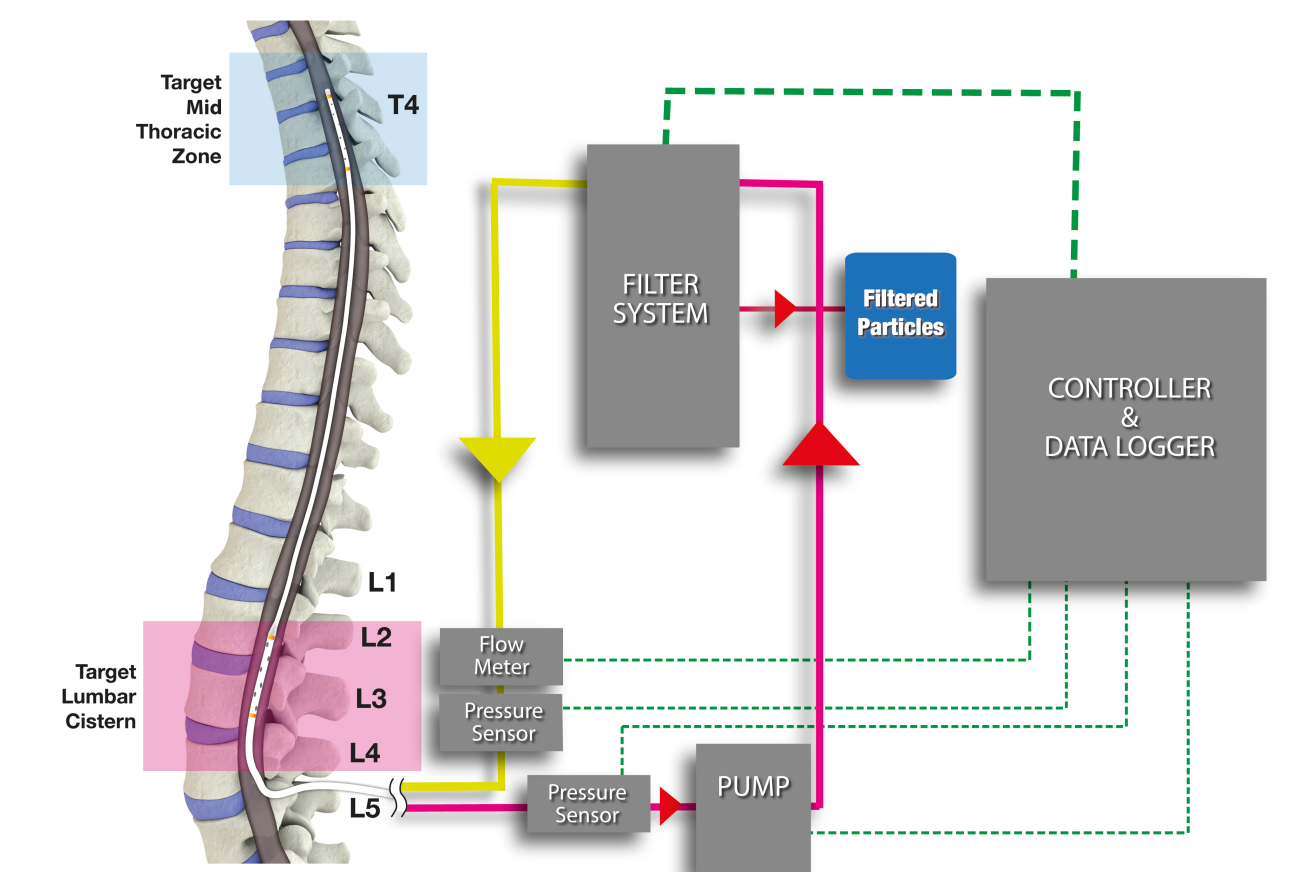


Figure 6. Human system schematic.

References and Acknowledgements

1. Gottfredsson M, Perfect JR. Fungal meningitis. *Semin Neurol.* 2000;20(3):307-322.
2. Williamson PR, Jarvis JN, Panackal AA, et al. Cryptococcal meningitis: epidemiology, immunology, diagnosis and therapy. *Nat Rev Neurol.* 2017;13(1):13-24.

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