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# Bactericidal Effects of Antibacterial Perfluorocarbon Ventilation

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# Summary of Presentation

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   II. Review of liquid ventilation
II. Methods
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# Background

# Background: Epidemiology

- Respiratory infection results in 500,000 hospitalizations, 110,000 deaths, and more than $13 billion in hospital costs per year
- Improved treatment is needed for cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and bronchiectasis patients with a bacterial infection
  - CF and COPD feature a change in mucus rheology that impairs mucociliary clearance, frequently leading to chronic infection and inflammation
  - Respiratory infections present in 55-60% of COPD exacerbations
  - 90% of US CF patients ultimately die of a respiratory infection

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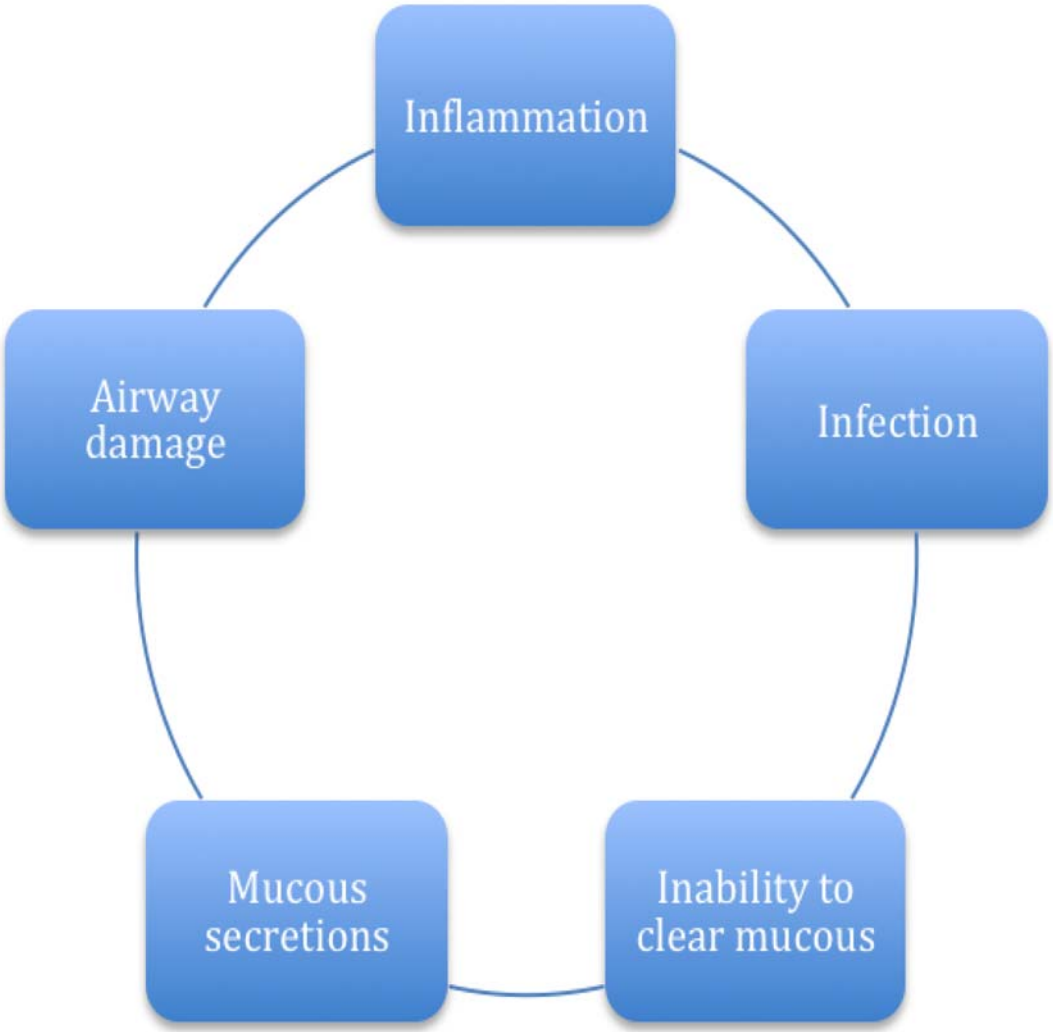
# A Chronic Cycle of Infection



# Background: Current Treatments

- Current treatments utilizing aerosolized and intravenous antibiotics have several shortcomings
  - IV antibiotics must be administered at high levels to achieve ideal concentrations at site of infection, often increasing risk of systemic toxicity
  - Aerosolized antibiotics require narrow range of particle size to reach peripheral airways
  - Aerosolized delivery cannot reach the poorly ventilated, most diseased lung regions

# Background: APV

- Antibacterial Perfluorocarbon Ventilation (APV) is a novel approach to pulmonary drug delivery that addresses the shortcomings of current treatment
- Lungs are filled with an emulsion of tobramycin in PFC and then ventilated for <2 hours
- Emulsion is composed of PFC, aqueous tobramycin and fluorosurfactant
- Emulsion allows gas exchange due to PFC's high solubility with $O_2$ and $CO_2$
- APV can improve current treatment in several ways
  - More uniform antibiotic distribution throughout the lungs
  - Removal of infected mucus
  - Anti-inflammatory properties inherent to PFCs

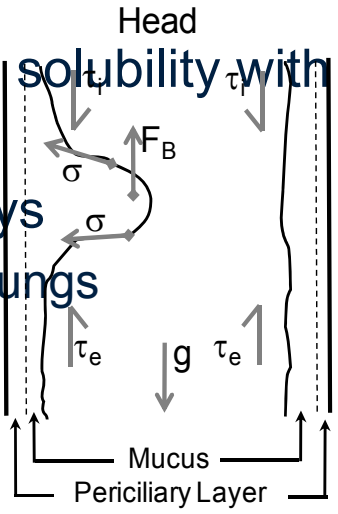



**Figure 1:** Airway depicting forces acting upon mucus. $\tau_i$ and $\tau_e$ are the shear stresses during inspiration and expiration, σ is the surface tension resisting mucus deformation, and $F_B$ is the buoyant force due to the density difference between PFC and mucus.

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# Methods

# Methods: *In vivo*

## Animal Experiments

- Inoculation
  - 200 µL inoculum containing approximately 106 colony forming units (CFU) of *P. aeruginosa* intra-tracheally delivered to each rat under sedation
  - Inoculum prepared by encasing mid-log growth *P. aeruginosa* in synthetic sodium alginate

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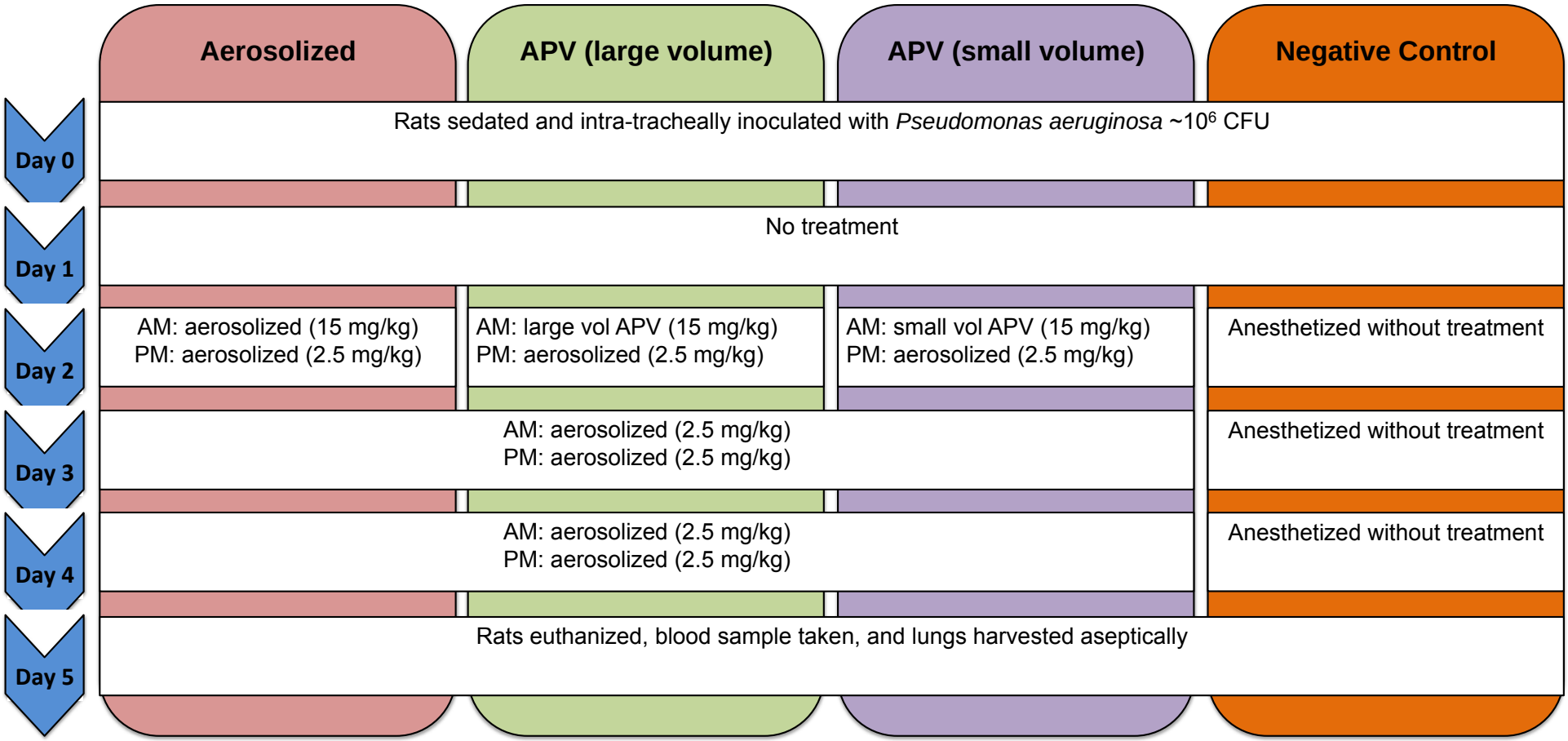
# Treatment Schedule



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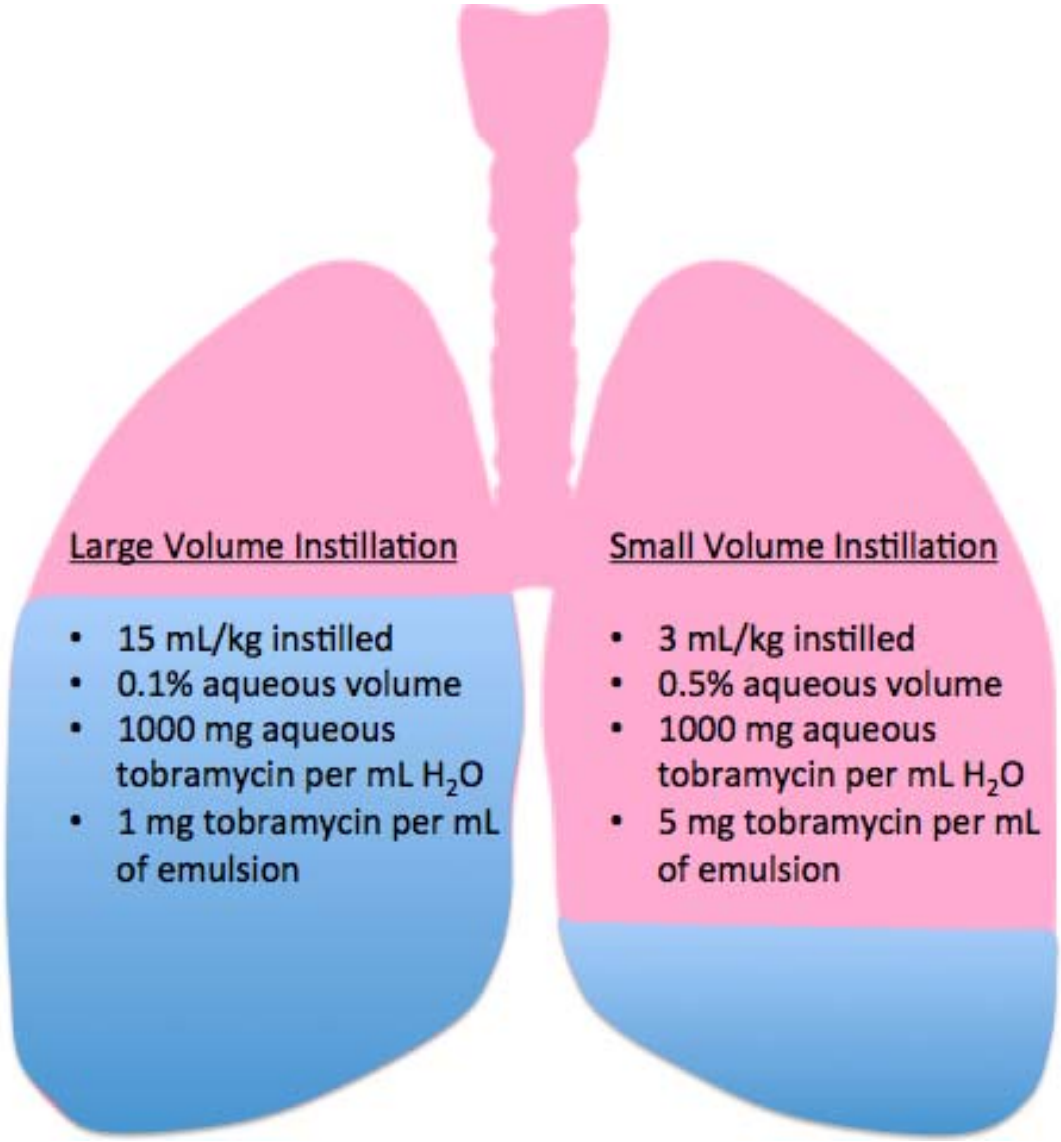
# APV Treatments



# Methods: *In vivo*

**Necropsy and Tissue Analysis**

- All rats were euthanized following three days of treatment (five days post-inoculation)
- The right lung was harvested aseptically and homogenized in sterile saline
- Homogenized tissue was serially diluted and cultured on cetrimide agar (a *P. aeruginosa* selective growth medium) in order to quantify bacterial load

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# Bacterial Cultures

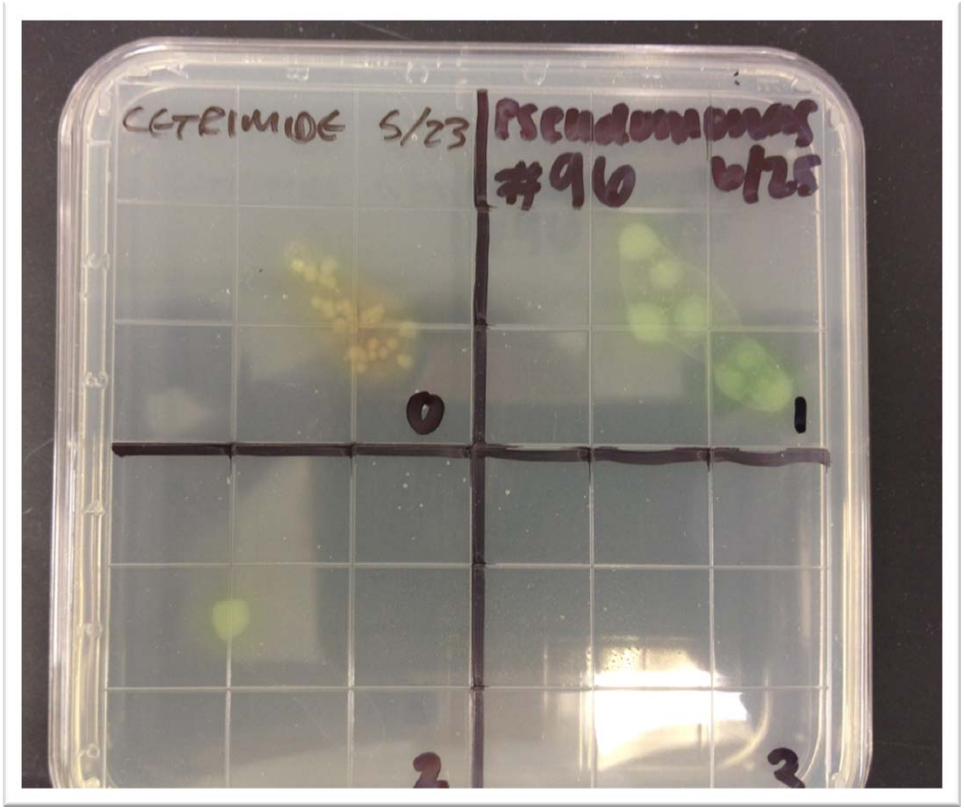



Figure 1: Bacterial load remained in the lungs post-treatment

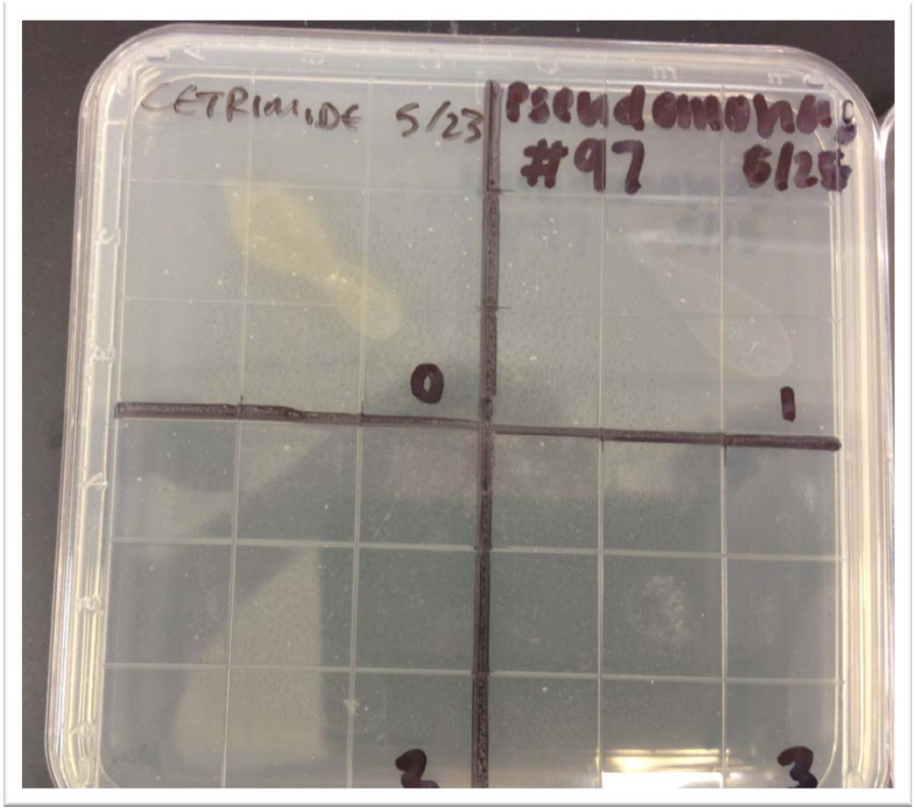



Figure 2: The treatment successfully killed all infection in the lungs

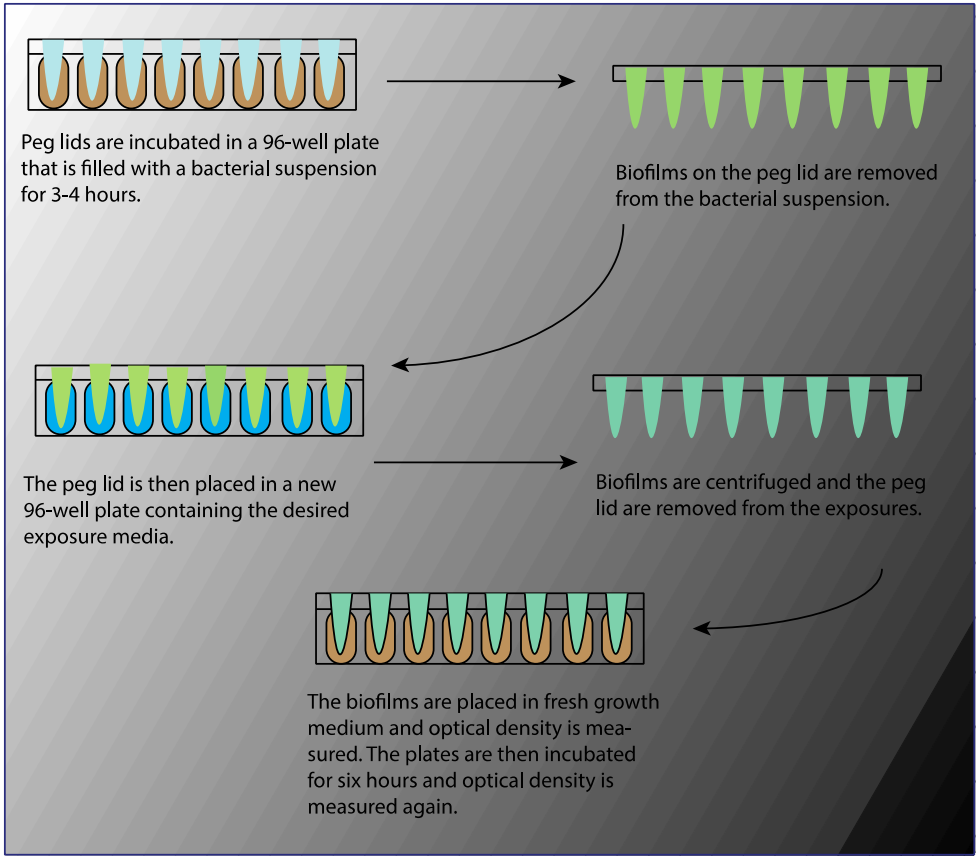
# Methods: *In vitro*

- **Biofilm Growth**
  - Biofilms were grown on plastic pegs by incubating a solution of mid-log growth *P. aeruginosa* in a 96-well plate with a peg lid for 18 hours

- **Exposures**
  - Biofilm-coated pegs were then submerged in new wells containing one of five exposure mediums (preoxygenated emulsion, preoxygenated PFC, unoxygenated PFC, unoxygenated emulsion, or growth medium) for a period of 2 hours
  - Preoxygenated groups were continually oxygenated throughout the exposure

- **Assessing Bacterial Growth**
  - Following exposure, adherent biofilms were centrifuged off pegs into new 96-well plates containing sterile growth medium
  - Bacterial growth was measured via change in optical density (600 nm) over

# Methods: *In vitro*



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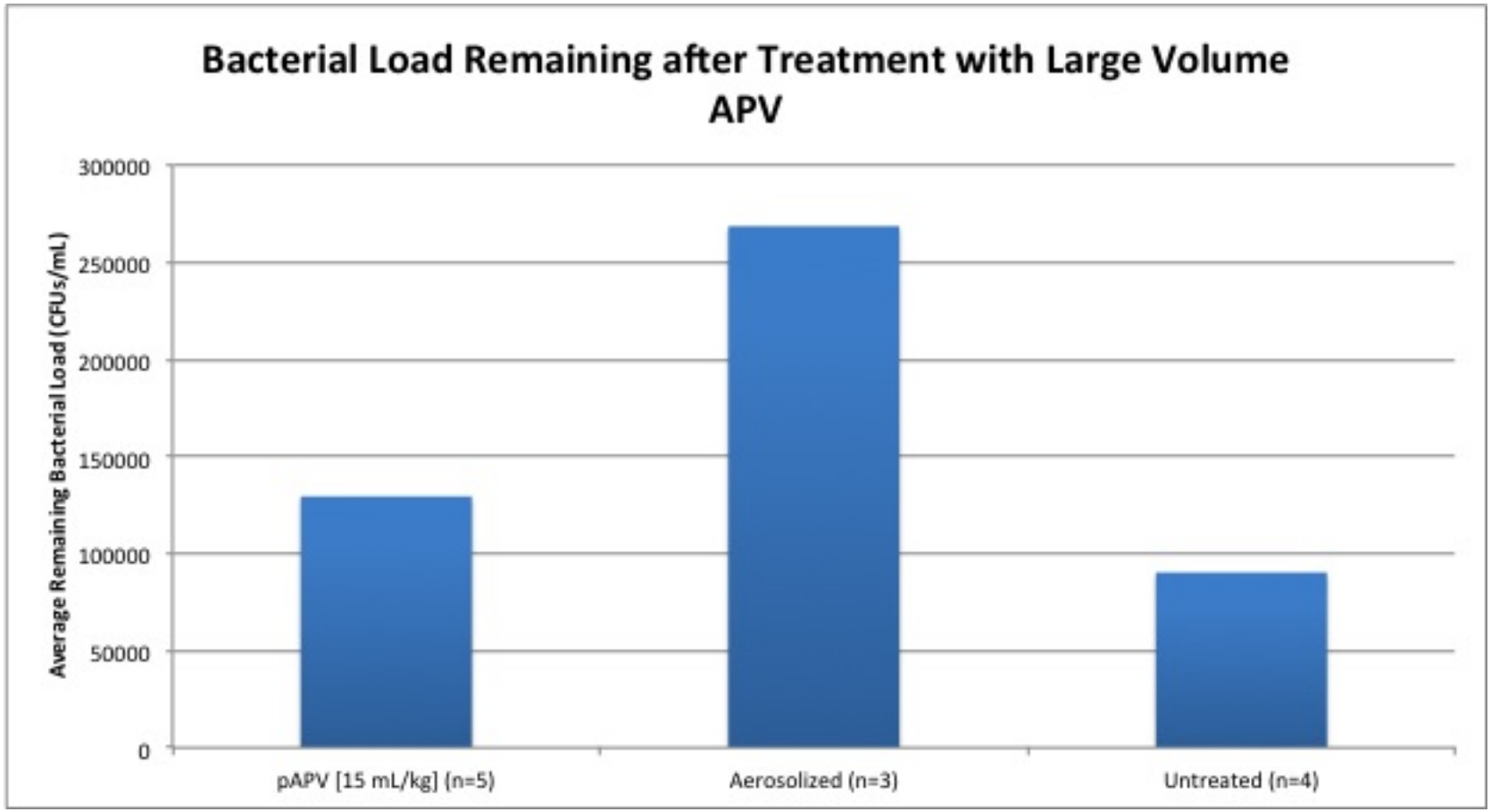
# Results

# Results: *In vivo*



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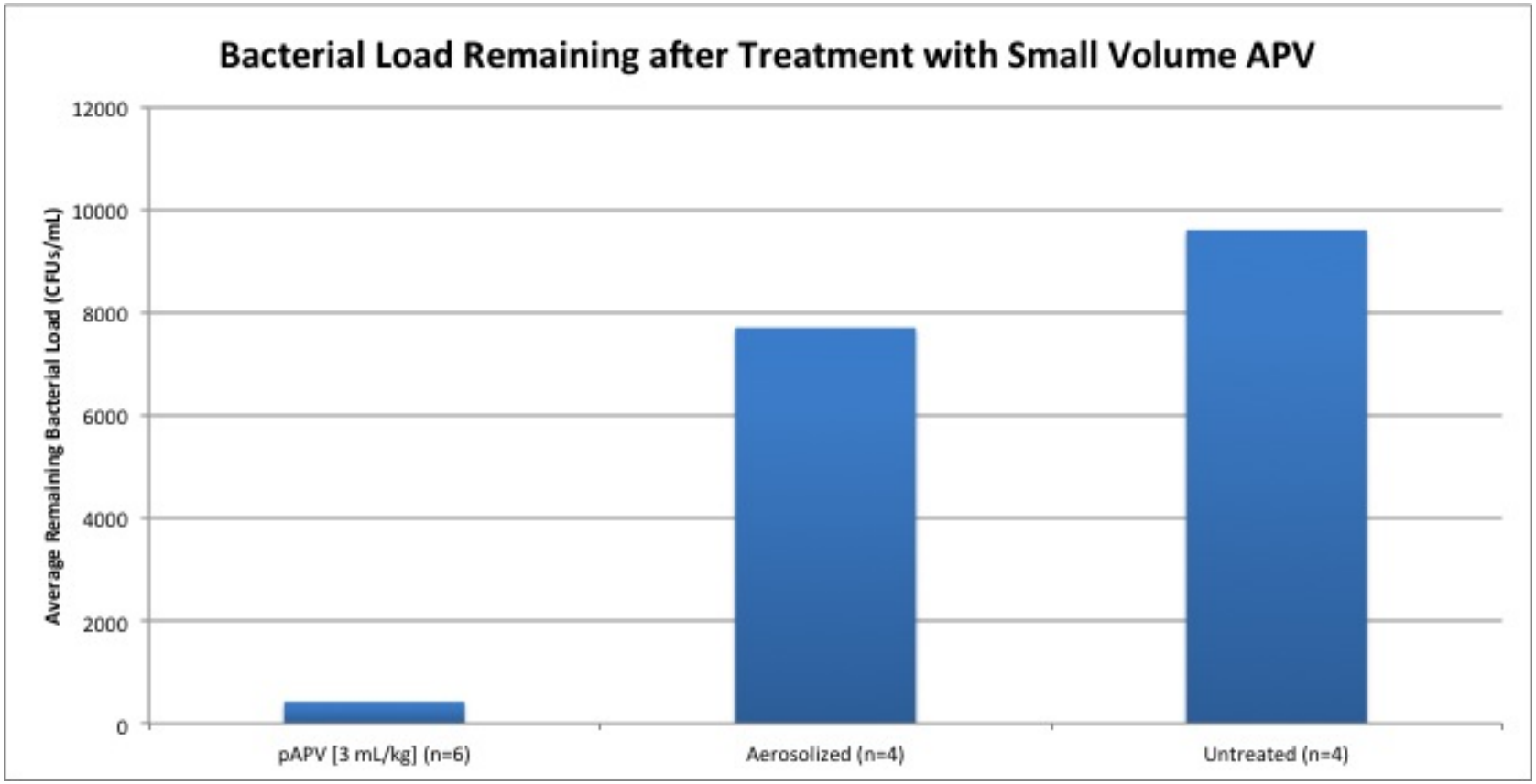

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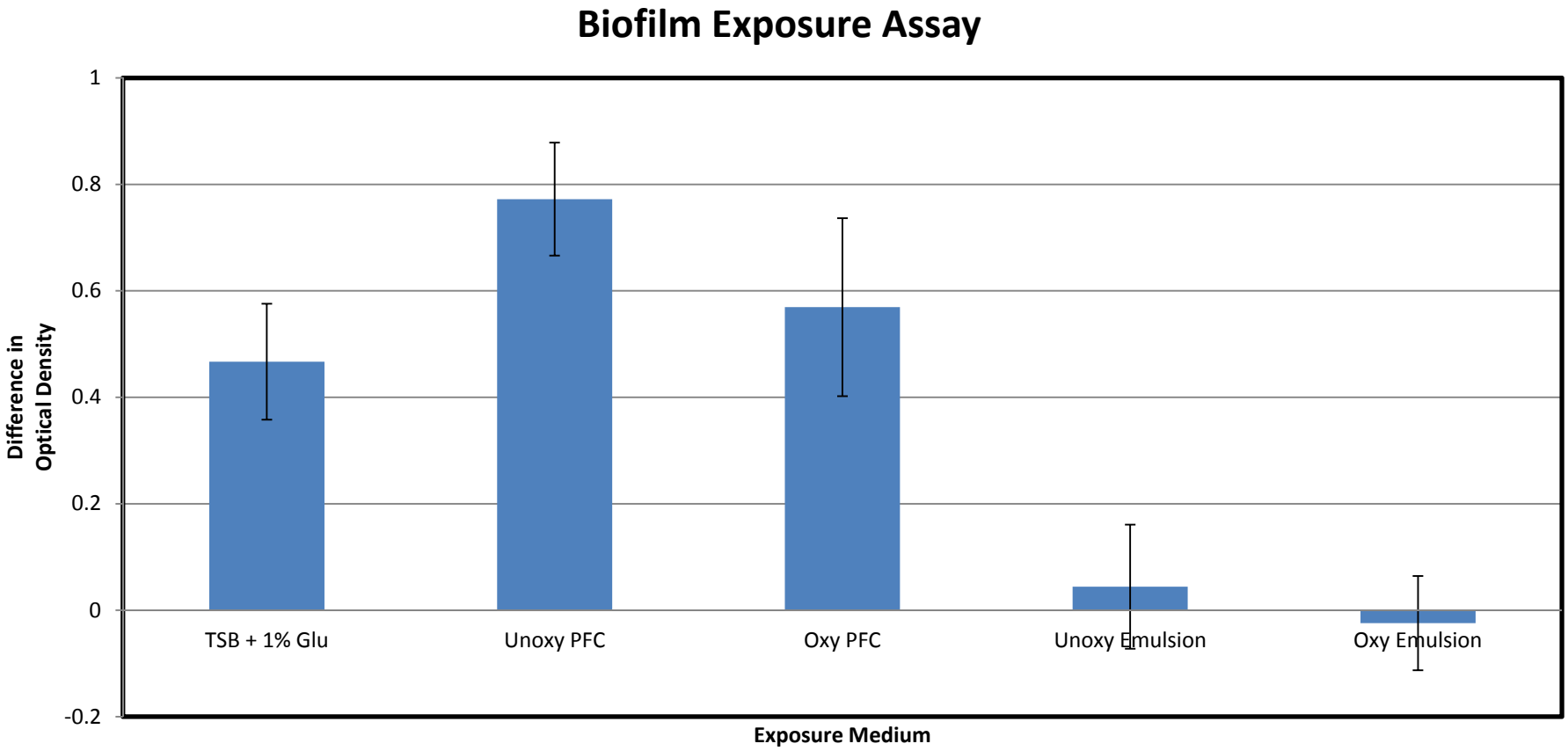

# Conclusions

# Animal Experiments

- Small volume APV results in lower remaining pulmonary bacterial load relative to aerosolized and large volume APV treatments
- We believe small volume APV performs better due to decreased volume of the PFC phase
  - PFC alone appears to promote bacterial growth (see biofilm exposure results), likely due to high O2 content
  - Over time, it is likely that the aqueous, drug-containing droplets will separate from the PFC phase and coalesce before all of the PFC is blown off, potentially leaving neat PFC in contact with the bacteria
  - This likely occurs to a greater degree during large volume APV
- Although more work must be done to optimize treatment, we have shown that APV is a viable means of pulmonary drug delivery with the potential for improvement over current treatments

# Biofilm Exposures

- The emulsion is capable of inhibiting biofilm growth in an *in vitro* setting
- Neat PFC promotes more bacterial growth than standard growth medium
- Oxygen content does not have a statistically significant effect on bactericidal ability of the emulsion


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